OPERATIVE DENTISTRY

Mar/Apr 2016
Volume 41
Number 2
117–228

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

OPERATIVE DENTISTRY

Volume 41 / Number 2
March/April 2016

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

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Vol 41/No 2 March/April 2016 Pages 117–228 OPERATIVE DENTISTRY ISSN 0361-7734

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Buonocore Memorial Lecture

Material Tissue Interaction—From Toxicity to Tissue Regeneration

G Schmalz • M Widbiller • KM Galler

Clinical Relevance

The topic of material tissue interaction includes different clinically relevant concepts ranging from the prevention of adverse reactions to the stimulation of regenerative processes.

SUMMARY

The topic of material tissue interaction has gained increasing interest over recent decades from both the dental profession and the public. The primary goal initially was to avoid adverse reactions after the application of dental materials. New laboratory test methods have been developed, and currently premarket testing programs, which attempt to guarantee a basic level of patient safety, are legally required worldwide. The dentist is responsible for selecting the correct indication as well as the proper handling of any newly emerging risk. Apart from this phenomenon-oriented "inert materials concept," the "analytical concept" focuses primarily on analyzing the reasons for adverse reactions, and identifying their associated modifying factors, in order to prevent them or to develop new and more biocompatible materials. The "concept of bioactivity" involves addressing the possibility of positively influencing tissue by materials application, such as the generation of tertiary dentin or antibacterial effects. Finally, tissue regeneration may be supported and promoted by the use of various suitable materials (matrices/scaffolds) into which stem cells can migrate or be seeded, leading to cell differentiation and the generation of new tissue. These new dental materials must also fulfill additional requirements such as controlled degradability in order to be suitable for clinical use. Clearly, the field of material tissue interaction is complex and comprises a wide range of issues. To be successful as dentists in the future, practitioners should remain informed of these important new developments and have the argumentative competence to both properly advise and treat their patients.

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DOI: 10.2341/15-249-BL

INTRODUCTION

Material tissue interaction has gained increasing interest over recent decades. In addition to new scientific findings and their clinical implications, this topic has attracted strong public interest (eg, the

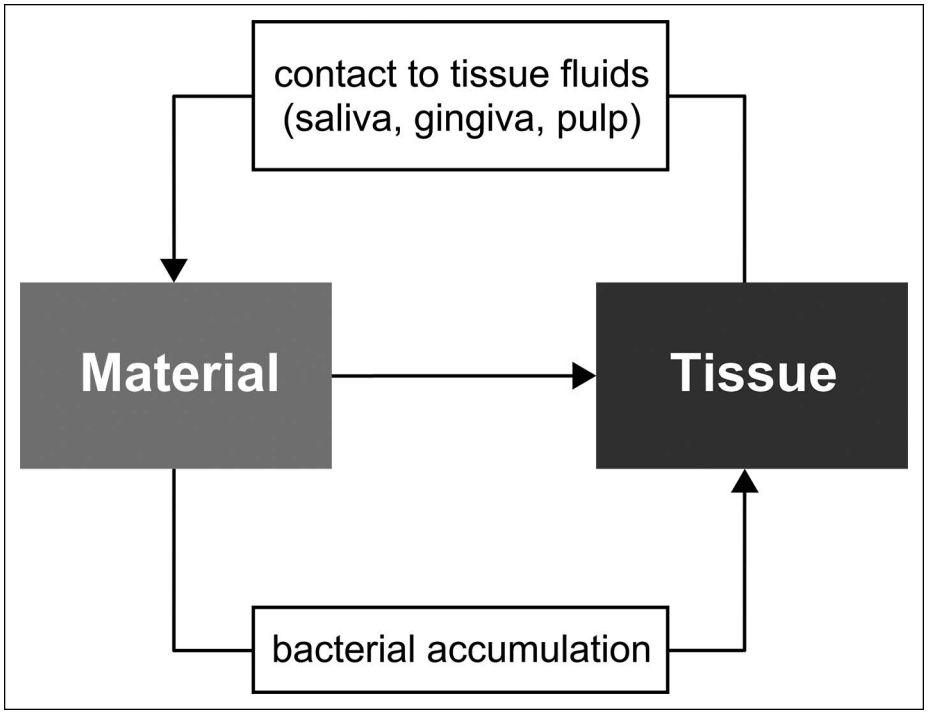


Figure 1. *Principle of material tissue interaction: material in contact with tissue fluids releases substances, which then act directly or indirectly (eg, via bacterial accumulation) on the tissue.*

discussion of mercury in amalgam and, more recently, the controversy over bisphenol A in resin-based composites).[1] Consequently, legal regulations concerning the safety of materials used in general medicine, including those used in dentistry, have been put into force.[2] Interestingly, dental research has been on the forefront of the medical field with respect to the development of tests, standards, and legal regulations related to material tissue interactions.

The basic idea behind such interactions is the fact that substances are eluted (ie, released) from the material when it comes in contact with various tissues, which then in turn influence the adjacent tissues (Figure 1). The process of release of substances from a material into the surrounding tissues is influenced by the material's bulk and surface properties, time after mixing (setting reaction), and the type of tissue. With setting materials (for example, resin-based composites) most substances (such as residual monomers) are released shortly after mixing.[3] Over time, different concepts regarding the material tissue interaction of dental materials have emerged. In this review, these concepts will be discussed, together with their practical legal and clinical consequences.

THE INERT MATERIAL CONCEPT

Definition

The inert material concept was the initial concept for material tissue interaction. The focus of this concept has been the attempt to avoid any tissue damage after the application of a dental material.[2] Since this goal is virtually impossible to achieve, the practical aim is to use biotolerable materials. Thus, biocompatibility can be regarded as the ability of a biomaterial to perform its desired function with respect to a medical therapy without eliciting any clinically significant adverse effects in the recipient.[4]

Tissues

Local reactions occur at the site of material application. Examples are pulp inflammation after pulp capping with dental adhesives (potentially followed by pulp necrosis)[5] and gingival inflammation after exposure to metal oxides from metal-ceramic crowns.[6] The mechanism may be the toxicity of substances such as metal ions released in toxic amounts (direct effect), but increased growth of bacteria (eg, on or under resin-based composites; indirect effect) may also play a role.[7] Recently, lip burning after the incorrect use of a high-power light curing unit through rubber dam has been reported.[8]

Systemic tissue reactions occur distant from the actual area of application. Resorption processes (passing the epithelial barrier), transportation in blood and deposition in organs, as well as metabolic transformation and excretion are the determining factors. In dentistry, systemic reactions occurred in the past if dentists polished or removed amalgam

fillings with high-speed rotary instruments but without water-cooling. As a result, symptoms of neurotoxicity, such as tremors, have been observed.[9]

Allergic reactions to dental materials can be regarded as the combination of local exposure and the activation of the body's immune system involving the activation of local dendritic cells and T cells in local lymph nodes.[9] Mostly type IV (delayed) and a few type I (immediate) reactions have been reported.[9] Clinical signs can be local, such as perioral eczema after the application of nickel-containing steel appliances, or can appear also in distant areas of the body (eg, after exposure of allergic patients to relevant metals). Examples will be described below.

Test Methods

Methods for testing adverse material effects include cell cultures, animal experiments, and clinical studies. One of the first studies on material tissue interaction was performed in dogs and tested the pulp reaction to silicate cement as compared to that associated with zinc phosphate cement. Even then the protective influence of residual dentin was already being described (see below).[10]

Modern test methods rely on cell cultures (screening tests) to determine unspecific (not related to the dental target tissues) damaging effects to cells (cytotoxicity, mutagenicity). Small animal tests, formerly called secondary tests, are performed, for example, to test for skin/mucosa irritation and systemic toxic effects but also for the potential of type IV sensitization to materials. Finally, usage tests are carried out where materials are applied in experimental animals as they would be in patients (eg, the pulp/dentin test to evaluate the pulp response to endodontic or restorative materials).[9]

Recently, special cell culture methods have been developed and aimed at replacing animal models; with these new models, a material is placed on one side of a dentin disk and a three-dimensional cell culture simulating the pulp tissue is placed on the other side of the disk (dentin barrier test).[11,12] A number of these test methods have been combined to establish standards (eg, ISO standards). The goal was to improve the comparability of data generated in different test laboratories as well as to save costs and test animals by avoiding duplicate testing.[2,13]

Legal Regulations

Legal regulations regarding the safety of dental materials were promulgated in the United States in 1976, followed by the European Union in 1993, and then by virtually all countries around the world. Accordingly, dental materials are classified as medical devices and must pass a certification process before they may be marketed. Although worldwide regulations differ in their details, the main aim of this certification process is the same, namely to demonstrate safety of the medical device. In this context, safety is defined as the absence of unacceptable risks. The manufacturers are legally responsible for the safety of their materials (see also the above definition of biocompatibility). In the United States, the Food and Drug Administration (FDA) is in charge of this process and provides detailed information regarding regulations on their Web site (http://www.fda.gov). The evidence of safety (biocompatibility) can be shown using the above-mentioned standards.

Despite the application of best practice test methods in this process of premarket safety evaluation, adverse reactions in the clinic cannot be ruled out completely, since a large number of patients under a variety of clinical conditions are exposed to the materials. Therefore, a postmarket surveillance/reporting system has been installed in each country, and each dentist is obliged to report adverse effects from dental materials to the respective authorities.

The aim of such legal regulations with respect to premarket certification is to protect the consumer, in this case the patient, from adverse effects. Finally, the manufacturers are responsible for the security of their materials. However, this legislation also protects the dentists: if they follow the indications as specified in the instructions for use provided by the manufacturer, then all patient claims can be directed to the manufacturer. However, significant responsibilities remain with the dentist.

Responsibilities of the Dentist

As mentioned above, the dentist is responsible (and legally liable) to follow the instructions for use and the indication, as specified by the manufacturer, for the individual patient. Adverse effects in patients from dental materials occur, in general, only seldom (<0.3%).[14] Allergies in patients are even more rare, but they exist.[9] In a group of contact dermatitis patients, 1.3% were allergic to bisphenol A diglycidyl ether dimethacryalate (Bis-GMA).[15] In a group of 86 patients in whom symptoms such as a burning mouth were attributed to dental alloys, 20% had a positive patch test reaction to a tested metal; however, this test was clinically relevant in only 5% of these patients, because the respective metal was part of an intraoral alloy. In a further 5% of the

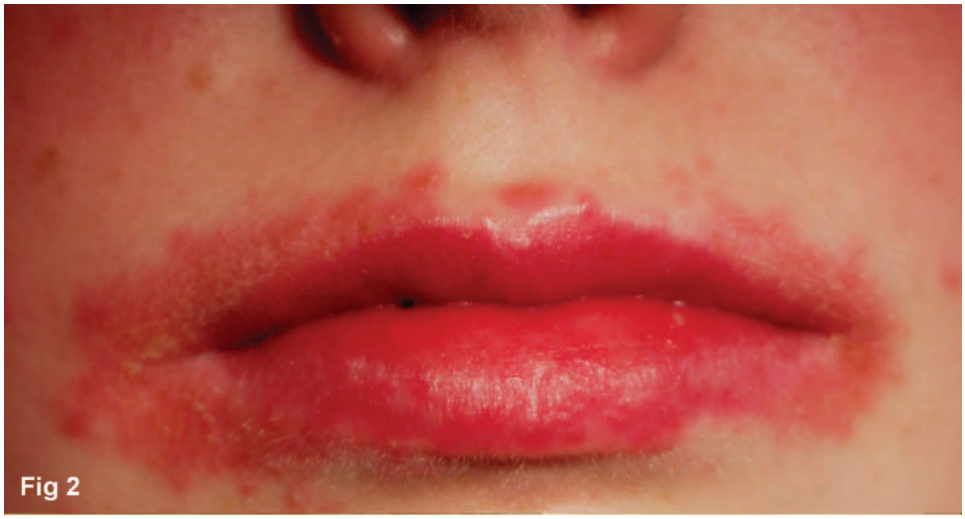
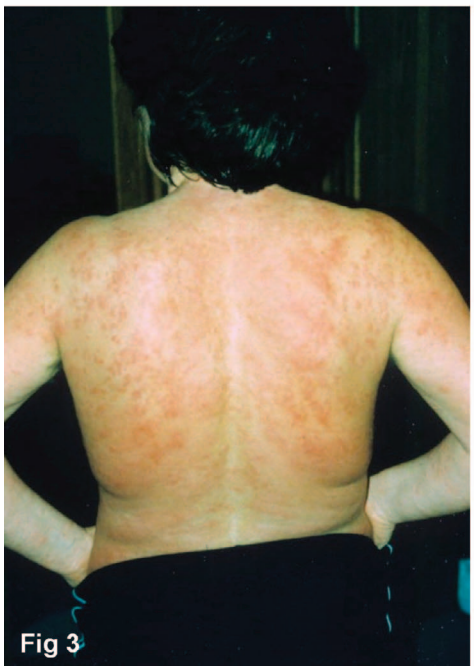
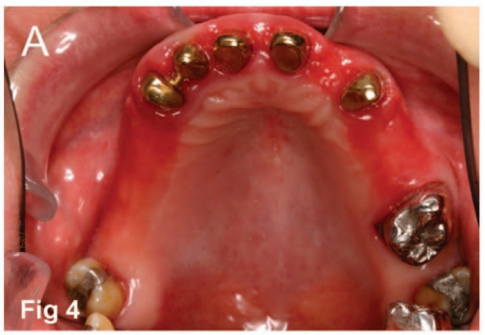


Figure 2. *Perioral allergic reaction in a 15-year-old girl after insertion of nickel-containing orthodontic wires (CuNiTi); patch test positive for nickel.*[9]

Figure 3. *Extraoral reaction in a 48-year-old woman after insertion of metal ceramic restorations; reaction subsided after exchange of the crowns with all-ceramic restorations.*[9]

Figure 4. *(A) Pronounced (not plaque-related) inflammation of the gingiva and the adjacent oral mucosa in a female patient with a positive patch test to gold, benzoyl peroxide, and hydroquinone. (B) Patient's prosthesis.*[9]





patients, the relevance was questionable.[6] Examples of allergic reactions are shown in Figures 2 through 4. However, allergies of dental personnel are more frequent. Based on a questionnaire study,[16] it was estimated that up to 2% of dentists showed allergic reactions to the monomers used in resin-based composites and related adhesives (Figure 5). Furthermore, it has been suggested[17] that exposure to methacrylates may induce cross reactivity to acrylates. The protection afforded against contact with relevant monomers by the use of gloves is only limited. Penetration of the monomers in commonly used solvents through latex or nitrile gloves takes place within a few minutes.[18,19] This means that any direct contact of the protected or nonprotected skin with resin materials (adhesives and resin-based materials) should be avoided (the so-called "no-touch" technique).[20]

Allergies to metals such as nickel are well known. However, there is also evidence that cross reactivity exists between nickel and palladium.[6,21,22] Hence, palladium-containing alloys should not be used in patients with a nickel allergy. Less known is the fact that there are allergic reactions to fragrances, such as essential oils, which include eugenol.[23]

Important for the dentist is to prevent any allergic reaction from occurring by conducting a correct anamnesis, at recall visits as well. In the event of clinical symptoms indicating the possibility of an allergy to dental materials, the dentist should refer

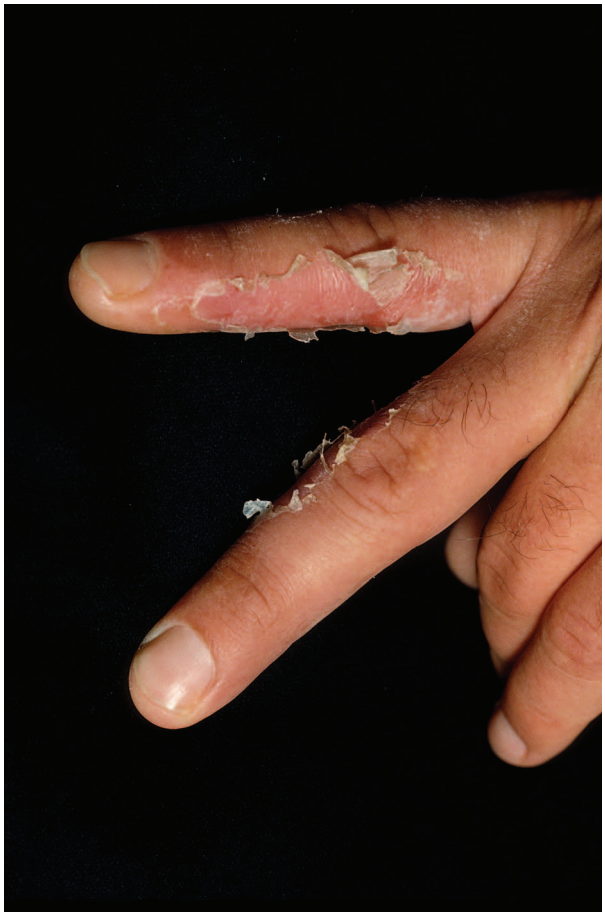


Figure 5. *Contact allergy to resin-based composites on the hand of a dentist.*[114]

the patient to a specialist for patch testing, which is the gold standard for confirming a type IV (delayed) allergy. The dentist should inform the specialist about the material that is suspected to have caused the allergic reaction and its composition. Patients may ask to have a patch test done before a dental treatment, even though they do not have symptoms indicating an allergy. This is usually not recommended, however, since there is a slight chance of sensitization by the patch test, and such a risk seems acceptable only if there are distinct clinical symptoms of an allergy. Furthermore, an allergy may develop at any time, even immediately after the test, and since no prediction is possible, patch tests do not necessarily provide any additional safety in this case.[24,25]

Localized lichenoid reactions (OLRs) have been described in the direct vicinity of amalgam, gold alloys, or composite resins (Figure 6A).[26-28] In cases with lesions restricted to the contact area with the material, 67.8% of patients showed a positive patch test reaction indicative of an allergic background. For lesions extending out of the contact area, this was the case in only 38.6% of patients.[29] Such lesions are normally attributed to an oral lichen planus (OLP), which is a dermatological disease (Figure 6B). Whitish lesions with striations (Wickham's striae) not related to a material are indicative of an OLP.[30]

Histology of OLR shows a clear line of inflammatory cells beneath the epithelium, which cannot be distinguished from OLP histologically. The etiology of the lichen is mainly unknown. The standard therapy for persisting localized contact lesions is to replace the contacting material. A patch test for allergy confirmation may be indicated. In case of persistence of the mucosal lesion, the patient should be referred to a specialist. Topical corticosteroids are the treatment of choice for OLP, although several other medications have been studied, including retinoids, tacrolimus, and cyclosporine, as well as photodynamic therapy.[30] OLP and OLR may be premalignant diseases, although malignant transformation has been reported to be below 1%.[30,31] In a

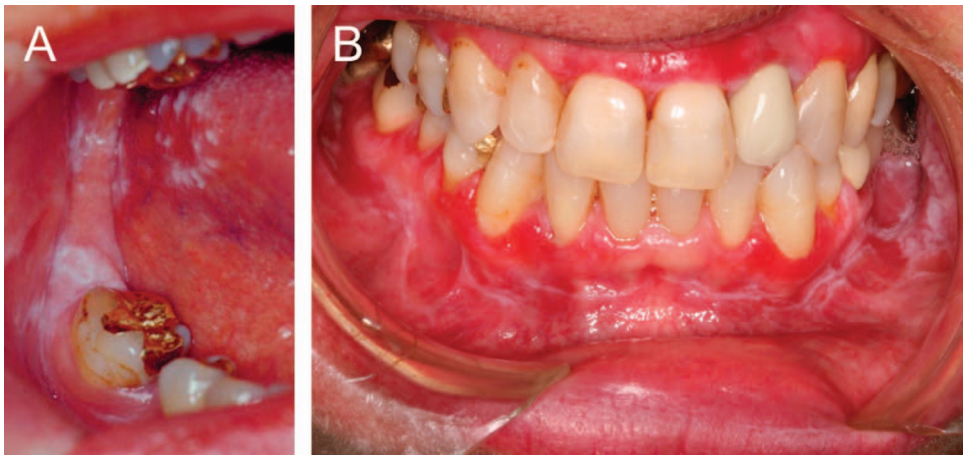


Figure 6. *(A) Localized lichenoid reaction of the mucosa contacting an alloy (OLR)*[9]*; (B) Nonlocalized oral lichen planus (OLP), not related to a restorative material.*[115]

further study,[32] four out of 192 cases of OLP developed into a squamous cell carcinoma. In any case, constant surveillance is required if the lesion does not subside.[32]

Off-Label Use

The "off-label use" term characterizes the application of a material or medication that is not approved for the specific indications listed by the manufacturer. Dentists are allowed to conduct off-label use, but in this case, the dentist alone, not the manufacturer, is responsible and legally liable for any adverse effect. This is the case, for instance, if a dentist uses adhesives for pulp capping and the manufacturer has not specified this particular indication for the product.

Newly Emerging Risks—Bisphenol A

Bisphenol A (BPA) has recently attracted considerable attention ("mercury of the 21st century"). Some authors[33,34] claim that BPA exposure leads to reduced fertility (male and female), irreversible changes of the developing organism (pubertal timing), neurotoxic effects, and other diseases, such as diabetes. In this context, dental resin materials such as fissure sealants are mentioned, and indeed, BPA is part of the molecular structure of commonly used dental monomers like Bis-GMA. However, BPA is not intentionally added to dental resin-based composites, and under physiological conditions conversion of the dental monomers into BPA has not been observed, with the exception of Bis-DMA, which was used in a few fissure sealants (eg, Delton®, DENSPLY Professional, York, PA, USA).[35-37] Unfortunately, the dentist is not able to determine whether a resinous material contains Bis-DMA based on the Material Safety Data Sheet, since manufacturers do not always provide such a detailed declaration of material components (see also the material related to referral for allergy testing, above).

However, BPA is used during the production process of Bis-GMA and related substances. Despite available purification processes, BPA residues (impurities) exist. Therefore, BPA is found in saliva and urine after placement of resinous materials (sealants and composite restorations).[38,39] This has only been observed shortly after placement, and values returned to normal after eight hours (saliva) or 30 hours (urine). Again, Bis-DMA products released 20 times more BPA than did Bis-GMA products.[38] Recent measurements of BPA release demonstrated that such levels of BPA are more than 2500 times less than the conservative BPA exposure limit, as proposed by the European Food Safety Authority, at 5000 ng per kilogram per day.[40] Therefore, according to present knowledge, the risk posed by the BPA content of dental resinous materials was rated to be negligible.[14]

THE ANALYTICAL CONCEPT

Whereas the inert material concept is mainly focused on the detection and description of the phenomenon of the adverse effect, the aim of the analytical concept is to elucidate the mechanism and the modifying factors underlying the phenomenon. The ultimate aims of the analytical concept are to improve or develop materials and to find ways to avoid adverse reactions elicited by existing products.[41] Test methods are generally more sophisticated than those used for the inert material concept; they include methods from microbiology as well as from biochemical and molecular cell biology, but also analytical chemical methods, in order to elucidate cellular pathways that are potentially altered by dental materials. Methods have to be chosen or tailored individually, whereas standardized test methods play only a minor role.

The Role of Residual Dentin and Bacteria

Dental adhesives are cytotoxic in direct contact with cells.[42] However, pulp reactions after the application of dental adhesives and composites in medium deep and shallow cavities do not occur if an intact dentin layer of >0.5 mm is present.[9] This shows that dentin may be protective against material toxicity. The reason is that the permeability of dentin is exponentially related to dentin thickness (Figure 7), in which the diameter of dentinal tubules increases with decreasing distance to the pulp.[43] Furthermore, after cavity preparation, dentin is covered with a smear layer, which further reduces its permeability.[44] In addition, the so-called dentin sclerosis or intratubular deposition of apatite reduces the penetration of potentially toxic substances. Some of these substances, such as eugenol or zinc from amalgam, become bound to organic and inorganic dentin components.[45,46]

However, pulp reactions to resin-based composites occur even with an intact dentin layer if bacteria are on the cavity floor; these bacteria may have invaded through (micro-) gaps between the material and cavity wall.[7] This means that dentin is not protective against bacteria and that bacterial penetration must be prevented by the correct application of an effective dental adhesive. Therefore, dental adhesives in medium deep and shallow cavities offer a

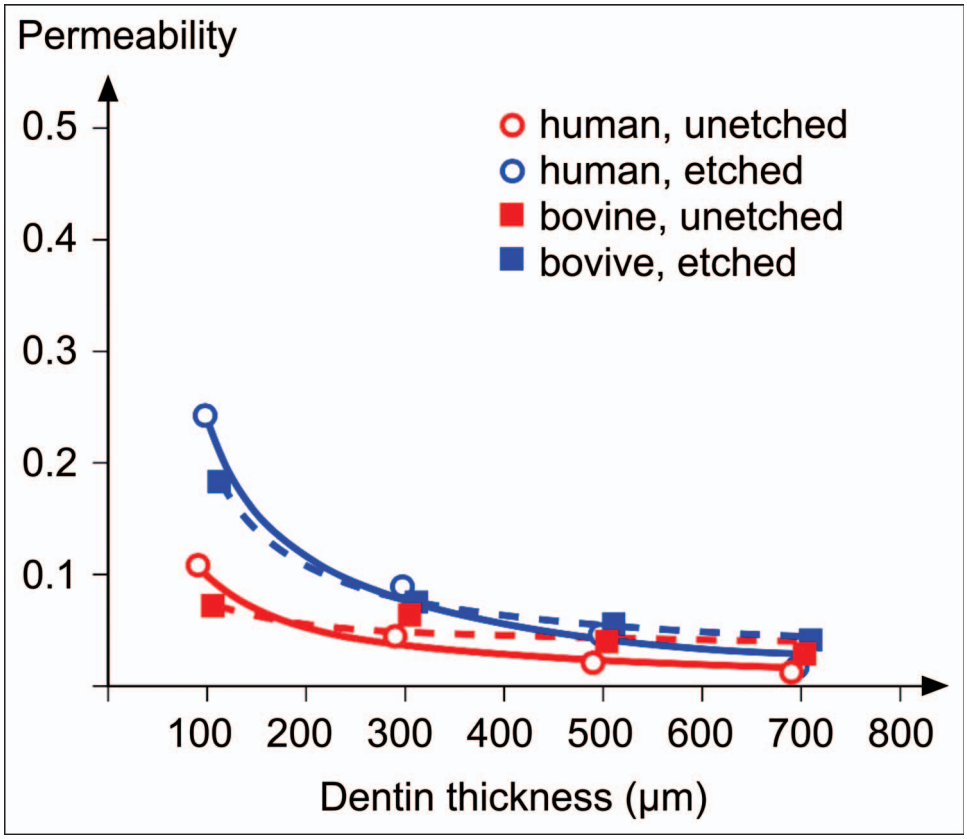


Figure 7. *Dentin permeability: a statistically significant increase in the permeability (here measured as hydraulic conductivity) was found only with very thin dentin discs.*[9]

means with which to prevent damage to the dental pulp and may be regarded as protective of pulpal health.

The Role of Materials

However, in deep cavities (<0.5 mm from the pulp) the situation is different. Pulp exposure is possible and difficult to diagnose when the patient is treated under local anesthesia with vasoconstrictors. In this case, direct contact of the unset material with the wet and living pulp tissue occurs. Contact with wet tissue may interfere with the material's setting process, which increases the release of substances from the material. Since there is apparently no, or only little, protection by the dentin, these cytotoxic substances from the material (eg, eluted monomers) may evoke cell necrosis, with the consequence of pulp inflammation, as seen histologically, which can occur even without any pain.[5]

Lower monomer concentrations do not cause cell necrosis, but rather apoptosis. Clinical studies[5,47,48] in humans provide evidence that pulp capping with dental adhesives leads to an inhibition of tertiary dentin formation (biomineralization). Such concentrations of relevant monomers, such as 2-hydroxyethyl methacrylate (HEMA) or triethylene glycol diemthacrylate (TEGDMA) cause DNA damage by the following mechanisms: cellular glutathione is consumed in the process of monomer detoxification.[49,50] As glutathione is also needed for maintaining the intracellular balance of reactive oxygen species (ROS), depletion of glutathione leads to an increase in ROS, which then results in oxidative DNA damage.[51] Subsequently, a series of adaptive cellular mechanisms is activated in order to maintain cellular homeostasis of the pulp tissue (eg, leading to DNA repair or to the disposal of damaged cells through apoptosis).[52] As a result of these highly energy-consuming reactions, the differentiation of dental stem cells into secondary odontoblasts, and thus tertiary dentin formation, is impaired. Interestingly, the use of certain antioxidants, such as *N*-acetyl cysteine, counteracts these effects and may be used as a component in future dental materials.[52,53]

Further studies[54,55] have shown that low concentrations of resin monomers such as TEGDMA or HEMA may lead to a reduced defense potential against bacteria. Cells of the innate immune system (here macrophages), which are in charge of clearing bacterial invasion through activating an inflammation process, are blocked by dental monomers at low

(noncytotoxic) concentrations. This may impair the immunological defense of the pulp against bacteria.

Clinical Consequences

In shallow and medium cavities, dental adhesives exert a protective effect on dental pulp; additional means of protection are not necessary. In deep cavities with the potential of pulp exposure, dental adhesives should not be placed directly on the dental pulp since they impair biomineralization and defense mechanisms against bacteria. Other bioactive materials (see below) should be used instead. Another promising approach, as a consequence of the above-mentioned reactions, is the incorporation of antioxidants (eg, *N*-acetyl cysteine) into monomers in order to reduce adverse cellular reactions, which is presently being investigated.

THE BIOACTIVE MATERIALS CONCEPT

The aim of the bioactive materials concept is to use a material as a therapeutic means to initiate specific biological processes, such as biomineralization or the introduction of antibacterial activity. However, the material itself stays, more or less, physically intact.

Induction of Tertiary Dentin Formation

The concept of inducing biomineralization is not new, since pulp capping with $Ca(OH)_2$ was introduced by Herrmann as early as 1928, although on a relatively empirical basis.[56,57] Recently, the mechanisms of tertiary dentin formation have been elucidated further. Apparently, signaling molecules that induce tertiary dentin formation, such as transforming growth factor–β1, are present in dentin and can be released through the application of $Ca(OH)_2$ or exposed on the dentin surface by ethylenediamine tetraacetic acid (EDTA).[58-60] Signaling molecules activate responsive cells (pulpal stem cells), which are present in specific niches in the pulp tissue. These cells migrate to the site of pulp exposure, where they differentiate into secondary odontoblasts and form tertiary dentin.[61-63]

In recent years, tricalcium silicate materials such as mineral trioxide aggregate (MTA) have been marketed. They release $Ca(OH)_2$ and induce tertiary dentin formation in animal studies, revealing slightly better results than $Ca(OH)_2$.[64,65] A large randomized clinical trial[66] has provided confirming evidence that MTA performs superior to $Ca(OH)_2$ after direct pulp capping. This was evaluated in a practice-based research network for up to two years.[66] The major problems with such materials are high costs, long setting times of up to several hours, and possible tooth discoloration, even after use of white MTA.[67] Therefore, MTA is mainly indicated for posterior teeth and can be used in combination with self-etch adhesives.

A different formulation of tricalcium silicate cement (Biodentine, Septodont, Saint-Maur-des-Fossés, France) was developed recently with a setting time of only several minutes.[68] The material releases $Ca(OH)_2$ and is less cytotoxic than MTA or glass ionomer cements.[69,70] It induces an upregulation of signaling molecules involved in biomineralization, such as osteocalcin (OCN), collagen type 1 (COL1A1), dentin sialophosphoprotein (DSPP), bone sialoprotein (BSP), and dentin matrix protein (DMP-1), in cell cultures and tertiary dentin formation in animal studies.[69,71-74] Furthermore, a diffusion zone between Biodentine and the adjacent dentin has been described.[75] Clinical studies on tertiary dentin formation are rare. A clinical trial comparing Biodentine with a glass ionomer cement (GC Fuji IX GP, GC Europe N. V., Leuven, Belgium) 12 months after application for indirect pulp capping showed no statistically significant difference regarding the clinical efficacy of both materials in patients with reversible pulpitis. However, cone beam computed tomography (CBCT) showed a significant difference where most healed CBCT lesions had received Biodentine, while most that did not heal had received the glass ionomer cement.[76] Direct pulp capping in premolars and molars scheduled for extraction showed similar results for MTA and Biodentine six weeks after application.[77,78]

Recently, a new material was marketed that contains both MTA and resin monomers (up to 50%) and is light cured (TheraCal LC, Bisco Inc, Schaumburg, IL, USA). This material releases more calcium *in vitro* than does a classical MTA material or a $Ca(OH)_2$ preparation and induces an alkaline pH similarly to MTA or $Ca(OH)_2$.[79,80] It apparently does not release $Ca(OH)_2$, and calcium release was less than for Biodentine.[68,81] The light-cured MTA was reported to be cytotoxic, but more detailed data on this finding were lacking.[82] In animal experiments on primates, this material stimulated bridging after pulp capping and elicited only a mild inflammatory response.[83] However, primate studies also showed bridging for resin adhesives,[84] which could not consistently be observed in humans.[5,47,48] Therefore, more investigation is needed in this area.

Antibacterial Materials

The use of antibacterial substances in dental materials has a long tradition. Mainly, substances such as metals (copper, zinc, or silver), fluorides, cetylpyridiniumchloride, different chlorhexidine compounds, glutaraldehyde, triclosan, zinc oxide, eugenol, and even antibiotics have been included in dental materials. Their antibacterial effect mainly depends on the continued release of antibacterial substances from the material.[41] The antibacterial monomer MDPB (12-methacryloyloxydodecylpyridinium bromide) was developed by Imazato and others.[85,86] This antibacterial moiety is tightly bound to the polymer network after polymerization. Surface inhibition of bacterial growth was shown *in vitro,* but the effect was masked after coverage with saliva proteins.[87] On dentin, the noncured primer in fact reduced the bacterial load.[88]

The main problem with this approach is that antibacterial substances are often also toxic to mammalian cells. However, there are strategies to prevent this: the above-mentioned antibacterial monomer is only active before polymerization; after curing the effect is reduced, which prevents damage to dental pulp.[89] Other strategies follow a similar idea of time regulation: photodynamic inhibition of bacteria (PIB) is based on light of a defined wavelength, which activates special antibacterial substances (photosensitizers). This is not a new approach, but as a result of the limited effectiveness of the photosensitizers used to date, it has not gained large market acceptance.[90] However, new photosensitizers with a higher efficiency have been developed; these photosensitizers may stand a better chance in the market in the future.[91,92]

These examples clearly show that dentists are able to actively influence cellular reactions by their choice of material application and that material tissue interactions have not only negative aspects (possible tissue damage) but also positive effects, such as the stimulation of repair or antibacterial action as well.

THE TISSUE REGENERATION CONCEPT

The aim of the tissue regeneration concept is to stimulate or induce the regeneration of tissues (eg, parts of the tooth like the dental pulp) by using a material that, in contrast to the bioactive material concept, is eventually replaced by the newly formed tissue. Tissue regeneration can be achieved using methods of tissue engineering, as described by Langer and Vacanti,[94] with the following components: 1) a matrix/scaffold, 2) signaling molecules, and 3) responsive cells (stem cells or progenitor cells*).

Signaling molecules, as mentioned above, are present in dentin and can be released (eg, by treatment with EDTA).[60] Furthermore, dentin surfaces can be activated and signaling molecules are exposed on the dentin surface, potentially inducing the differentiation of pulp stem (progenitor) cells contacting dentin to become secondary odontoblasts (contact differentiation).[95] Stem cells are present not only in the pulp tissue but also in periapical niches and in inflamed apical tissues as well as in the evoked intracanal blood influx from periapical tissues.[96-98] According to the tissue engineering concept, a matrix/scaffold is needed, into which signaling molecules and stem cells are incorporated. To date, two concepts have been discussed in the literature: one is the isolation of stem cells with *in vitro* expansion for reimplantation; the other is a primarily cell-free concept, which relies on the immigration of resident stem cells into a matrix/scaffold as a result of the presence of signaling molecules (cell homing).[96,99] Both concepts rely on a suitable matrix/scaffold that can be regarded as a dental material, which must have properties different from commonly used dental materials.

A New Group of Dental Materials

In contrast to commonly used dental materials, these matrix materials should undergo controlled degradation in order to be replaced by new tissue.[100] The incorporated signaling molecules should be released in a prolonged way, and heparin was shown to be able to control this process.[101] Furthermore, not only the material itself, but also the degraded components, should not be toxic to the target cells. Specifically for pulp regeneration, the matrix material should be injectable.[100] A number of candidate materials are available (Table 1), most of which are hydrogels offering a tissue-like water content.[100]

The possibility of pulp regeneration has been shown in several studies. One of the first was presented by the group of J. Nör,[102] who placed dentin slices filled with a solid poly-L-lactic acid matrix containing human pulp-derived stem cells and vascular endothelial growth factor subcutane-

* The term "stem cells" for undifferentiated cells in certain areas of the tissues (niches) is not correct for such cells in the dental pulp. The term progenitor cells is more appropriate, since such cells in the dental pulp, which have the potential to develop into secondary odontoblasts, already show a certain degree of specialization. However, the term "pulpal stem cells" is often used in the literature.[93]

Table 1: *Scaffolds for Pulp Regeneration*	
Natural	**Synthetic**
Collagen	Polyethylene glycols (PEGs)
Alginate	Self-assembling peptides
Fibrin	

ously into immunodeficient mice. After six weeks, a pulp-like tissue with odontoblast-like cells at the dentin surface had developed.[102] In addition, *de novo* formation of new tubular dentin was shown in this model.[103] Similar results were observed in a tooth root model after injection of a hydrogel material (self-assembling peptide) together with human pulp-derived stem cells and signaling molecules. A pulp-like tissue was generated six weeks after implantation in immunodeficient mice if the dentin had been conditioned with EDTA prior to cell seeding (Figure 8). The sole usage of sodium hypochlorite led to dentin resorption.[95] Other experiments using full-length roots of human premolars filled with Puramatrix (BD Biosciences, Bedford, MA, USA) or recombinant collagen and stem cells from primary teeth showed similar results.[104] Studies in dogs[99] also showed new pulp tissue formation in teeth: after extraction, apectomy, and removal of pulp tissue, the root canals of the respective teeth were filled with collagen as a matrix, cells, and signaling molecules. Fourteen days after replantation, newly formed pulp-like tissue was detectable, and odontoblast-like cells had formed on the dentin surface.

Clinical studies are under way, but results are not yet available.[105] Case reports[106,107] on necrotic teeth with open apices have been published and showed healing of periapical lesions with continued root formation after a so-called revascularization protocol. However, clinical success was only determined by radiographs and vitality testing. One case allowed for later histologic evaluation: a pulp revascularization protocol had been applied to an immature permanent incisor with irreversible pulpitis and without apical lesion. The tooth fractured 3.5 weeks later and had to be extracted. Histology showed a loose connective tissue similar to pulp and a layer of flattened odontoblast-like cells lined along the predentin.[108] One can speculate whether this new tissue was due to regeneration or was a remnant of the original pulp. In another case report[109] it was shown that soft connective tissue, similar to that in the periodontal ligament and cementum- or bone-like hard tissue, formed in the canal of a human revascularized/revitalized tooth.

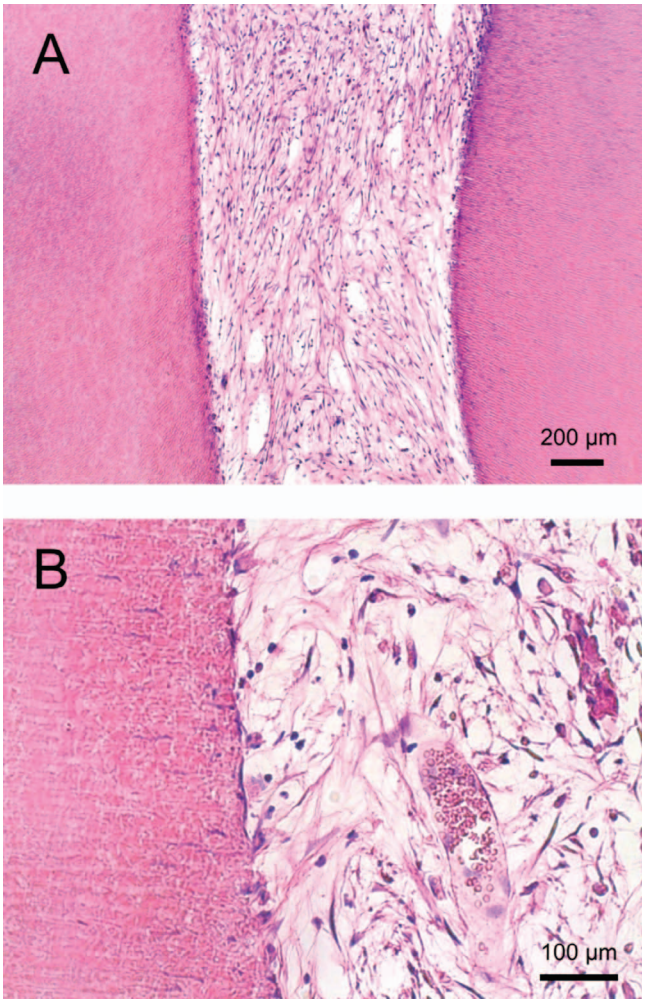


Figure 8. *(A) Dentin cylinders show formation of connective tissue after pretreatment with EDTA, while no resorptions are observed (hematoxylin and eosin). (B) A pulp-like tissue has formed and blood vessels are visible. Cells are in close contact to dentinal wall and showed a flat morphology (hematoxylin and eosin).*[95]

Although some of these data are promising, a number of serious problems still exist.[110] Infection and its control are apparently a major concern.[107,111] Furthermore, it is still unclear whether true regeneration takes place with the formation of pulp or pulp-like tissue and dentin or whether it is instead the repair and formation of connective tissue, bone, and cementum.[109,112] Additionally, it is not yet clear which of the two concepts, cell transplantation or cell homing, will ultimately be the most clinically successful. Clearly a better understanding of the characteristics of pulp-derived stem cells is necessary.[113] Nonetheless, advances in this area have been rapid, and the main focus lies firmly on the development of a suitable material.

PERSPECTIVES

Concerning the current concepts delineated in this review, the inert material concept will be further developed in the future but will remain mainly a routine testing method. The analytical concept will continue to attract major scientific attention since the understanding of material tissue interaction will be the basis for further material improvement, as emphasized by the recent report of the European Commission on the future of restorative materials.[14,41] The bioactive concept as well as tissue engineering will play major roles in the future as regenerative medicine advances.

A main challenge will be combining these concepts (eg, the inert material concept with concepts of bioactivity or tissue engineering). Possible solutions include the following:

1. Adjustment of the concentration of active substances in a material or eluted from a material to elicit the desired effect, but with concomitant avoidance of cell toxicity (window of effectivity). However, bacteria are normally more resistant than cells, and this approach may thus be problematic.
2. Development of materials that release antibacterial substances into their vicinity; however, as a result of low diffusibility of such substances in the tissues, they should not reach further distant cells in toxic amounts. An example is eugenol, which, when mixed with zinc oxide, is released in high doses on the cavity floor and thus elicits an antibacterial effect. However, as a result of the restricted diffusion of this hydrophobic substance through the hydrophilic residual dentin, it does not damage dental pulp cells if the residual dentin layer is thicker than 0.5 mm.
3. Controlling the time of action by adjusting a material so that the active substance is only released in the unset state or briefly after setting. This effect can, for example, be observed with an antibacterial monomer (see above). A different way to control the time of action is to apply antibacterial (and potentially cytotoxic) root canal dressings for only a limited time period of just a few days. An additional method for controlling the time of action is PIB, which was also mentioned above.

In any case, material tissue interaction is not only an important area of research and development but it also has direct influence on our daily practice. The modern patient who seeks information from many easily available sources will ask his dentist for advice. Therefore, the successful dentist of the future will not only be the expert who knows all relevant diseases and how to prevent or treat them but will also be someone who possesses the argumentative competence to effectively communicate with a well-informed patient and to participate in public discussions.

Conflict of Interest

The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

(Accepted 27 August 2015)

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Clinical Technique / Case Report

Prosthetic Rehabilitation of a Patient With Gastroesophageal Reflux Disease: Five-Year Follow-up

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FO Araujo

Clinical Relevance

This article discusses the different levels of clinical manifestation of acid erosion and indicates that veneers may be a promising alternative for restoring esthetics and function when combined with an appropriate medical diagnosis and clinical follow-up.

SUMMARY

Tooth wear is a multifactorial process that is a growing concern in dentistry. This phenomenon can be caused by mechanical (attrition, abrasion, or abfraction) or chemical (erosion) processes. Etiologic factors in dental erosion can be due to changes in behavior, an unbalanced diet, or gastrointestinal disorders such as acid regurgitation, which may influence the salivary flow rate and buffering capacity of saliva. This case report describes an esthetic rehabilitation of a patient with gastroesophageal reflux and dental erosion, with a treatment rationale that includes the use of a diagnostic template and five-year follow-up. This technique, presented here in a clinical case with moderate enamel loss, integrates an additive wax-up and a direct intraoral bis-acryl resin mock-up. Lithium disilicate glass-ceramic (IPS e.max Press, Ivoclar Vivadent) laminate veneers were fabricated with the heatpress technique. They were veneered with a layering ceramic (IPS e.max Ceram, Ivoclar Vivadent) to improve the appearance of the incisal edge. The case demonstrated the success of veneers as an effective, conservative, and esthetic treatment for patients with this pathology.

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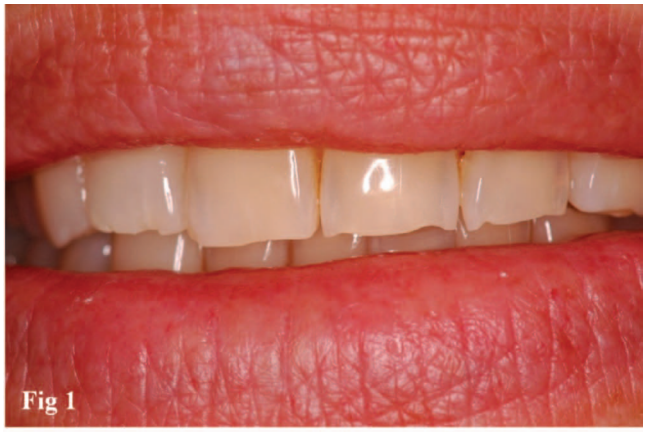
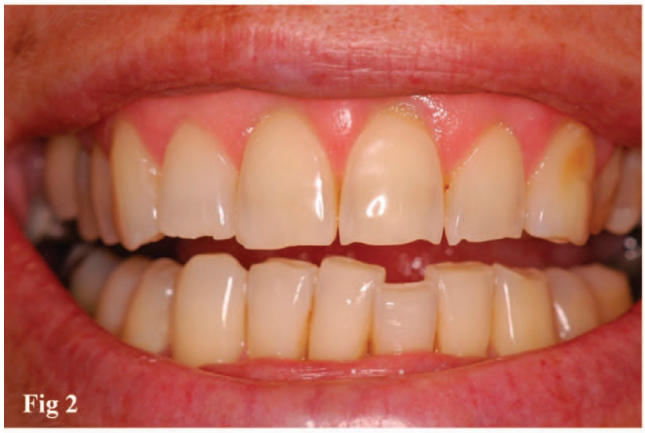
DOI: 10.2341/14-297-O

INTRODUCTION

The incidence of patients presenting dental wear has represented a growing concern for dentistry. Dental experts have indicated over the last decade that there has been an increased prevalence and severity of wear caused by the process of dental acid erosion.[1-4] Other studies have reported that the cause of tooth wear is multifactorial, or a combination of erosion, abrasion, and attrition.[2,3,5-7]

Early forms of erosive wear can be neglected because there are few or no symptoms or signs.[3,4]





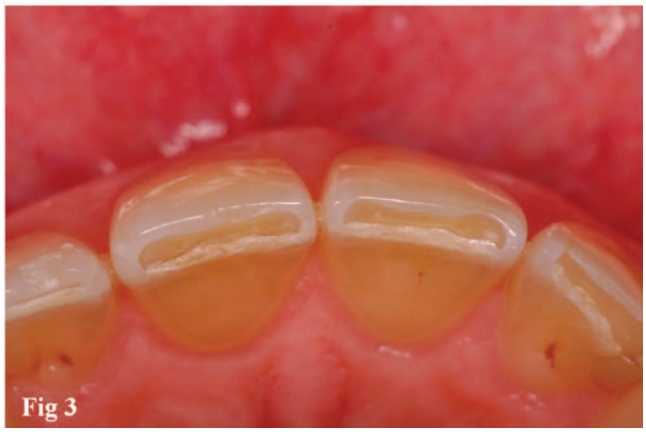


Figure 1. *Initial appearance.*
Figure 2. *View of incisal and vestibular tooth structure loss.*
Figure 3. *View of initial palatal and incisal wear.*

Thus, it is necessary for a dentist to carefully evaluate the clinical characteristics of the lesions to establish the diagnosis of dental erosion. In most cases, patients do not seek treatment and only discover an issue at a more advanced stage where the teeth are already sensitive or the esthetics are compromised. This is a common symptom in patients with anorexia nervosa or bulimia.[3] Gastric reflux directly affects the lining of the esophagus and is responsible for dental erosion. Gastroesophageal reflux disease (GERD) affects all age groups.[1,8]

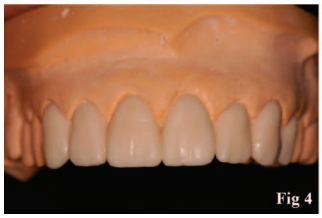
Dental erosion may be defined as the loss of tooth structure due to a chemical process that does not involve bacterial action and may be multifactorial in origin.[1,2,5,7,9] Causes can be intrinsic (eg, gastric) or extrinsic (eg, acidic foods or drinks).[6,7,9] Behavior changes, an unbalanced diet, various drugs, and acid regurgitation can influence the composition and buffering capacity of saliva, with these etiologic factors considered for dental erosion. Acidic foods are a common part of the modern diet, particularly fruit acids. The frequency of consumption of acidic foods and beverages plays an important role in dental erosion. Saliva may provide some protective benefits, particularly through the clearance and neutralization of acids.[2,9,10] Patients who present with GERD present a diminished capacity to buffer saliva and a higher prevalence of tooth wear, especially on the palatal aspect of their teeth. This is the retrograde movement of gastric juices into the esophagus, reducing the pH of the oral cavity and causing irreversible loss of minerals in the enamel surfaces, and can result in inflammation of the oral mucosa.[11]

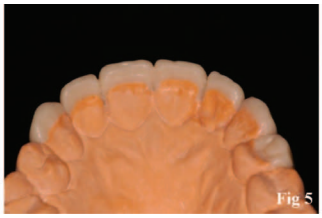
Various treatments have been described to treat patients with tooth erosion. The choice of treatment should be planned, which initially must be performed using a diagnostic wax-up to determine appropriate treatment.[12,13] The restorative options include the following: direct composite resin and indirect partial and full ceramic restorations, with the goal of using the most conservative approach possible.[14] The present study describes a case report of esthetic rehabilitation of a patient with GERD and dental erosion, with a five-year follow-up.

CLINICAL CASE

A 50-year-old female patient (STC) was admitted to the dental clinic at the University Tuiuti of Parana reporting a diagnosis of GERD a few years ago and dissatisfaction with her smile. Upon clinical examination, there was wear on the incisal and buccal surfaces of her anterior teeth caused by acid erosion. A radiographic examination, study models, and photographic documentation of the clinical case were made (Figures 1 through 3). The patient was referred for medical evaluation, undergoing hiatal hernia surgery.

Based on the diagnostic data and initial esthetic evaluation, wax-up of her maxillary anterior teeth (Figures 4 and 5), which was created freehand,





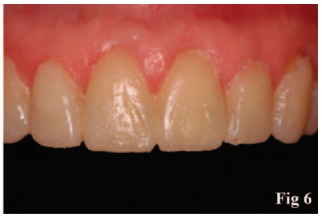
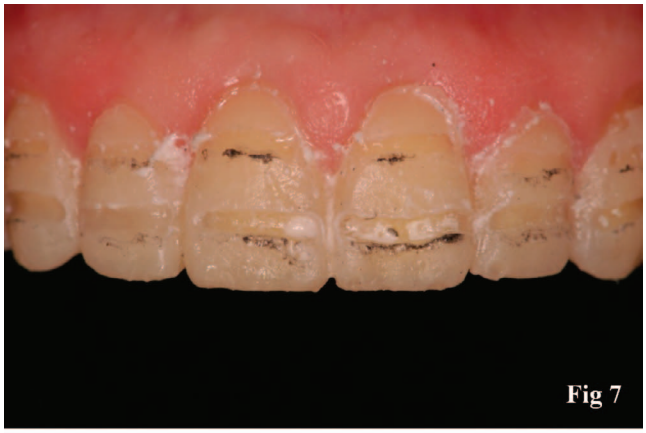
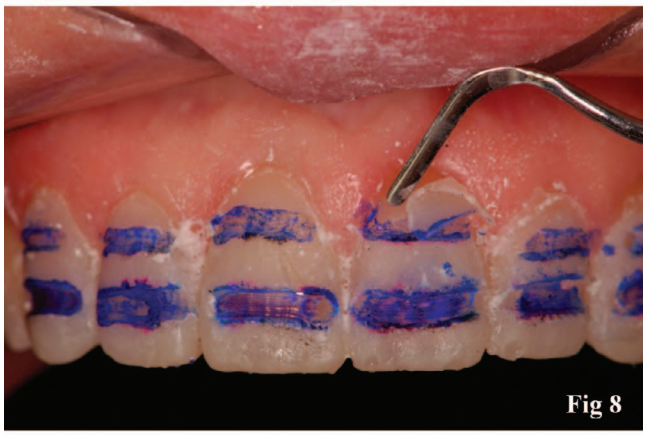


Figure 4. *Diagnostic wax-up of the upper jaw.*
Figure 5. *Palatal view of the diagnostic wax-up.*
Figure 6. *Bis-acryl resin mock-up.*





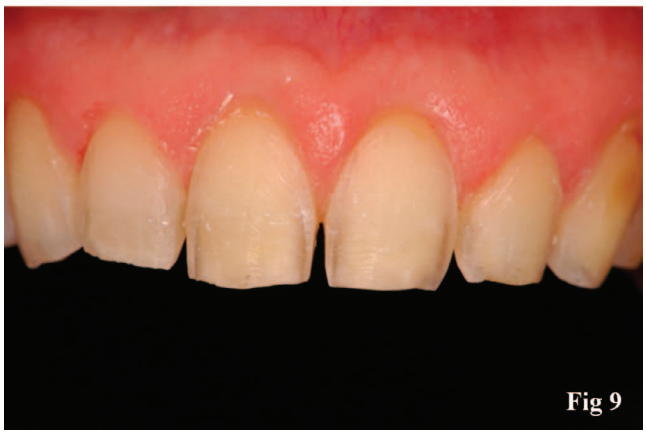
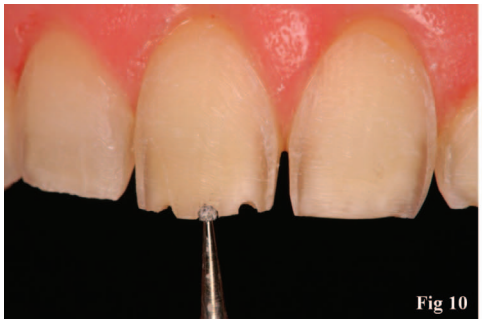


Figure 7. *Groove guidance made in the mock-up to a depth that represents the final thickness of the porcelain veneer and allows for the variable thickness of enamel on the eroded surface.*
Figure 8. *Removing the mock-up after completing the orientation grooves.*
Figure 9. *Preparations of the buccal face.*

served as a baseline for the initial diagnostic evaluation for the patient. The silicone index, which was made on the cast, was filled with Bis-acryl resin (Structur, VOCO GmbH, Cuxhaven, Germany) and adapted to maxillary anterior teeth with finger pressure until the material was fully polymerized (Figure 6). Excess polymerized resin was removed with a blade. A thin layer of acrylic resin was visible on gingival tissues and was removed with a blade.

When the patient returned, preparations were performed with care (Figure 7), because the teeth had already lost tooth enamel. Two horizontal grooves were prepared on the buccal surface of the mock-up, with depths varying from 0.5 to 0.7 mm, using a bur with a diamond tip. The grooves were cut to a depth that represents the final thickness of the porcelain veneer and allows for the variable thickness of enamel on the eroded surface. Then, two marks with pencil on the mock-up were drawn to aid in preparing two grooves. The marks were measured with a millimeter probe for evaluation of the depth of the preparations (Figure 8). The mock-up was removed, and the buccal surfaces were reduced using a #4138 diamond bur (Jota, Rüthi, Switzerland), according to the restorative plans (Figure 9). The cervical margin was exposed using #00 retraction cord (Ultrapack, Utradent Products Inc, South Jordan, Utah, USA), and the gingival margins of the porcelain veneers were set at the cervical gingival level. The incisal reduction was set at 1.5 mm using guiding grooves (Figures 10 and 11). This step was performed to facilitate insertion of the veneer and color stratification in this area by the prosthetic technician. The preparation was finished using ultra-fine diamond burs (Jota) and Soft-Lex discs (3M ESPE, St Paul, MN, USA) at low speed.

With the preparations completed (Figure 12), an impression of the maxillary arch was made using a condensation silicon impression material (Speedex, Vigodent, Rio de Janeiro, RJ, Brazil). Provisional restorations (Structur 2QM, VOCO GmbH) were cemented after the tooth preparation procedures. Cementation of the relined acrylic resin provisional restorations was done using a noneugenol temporary cement (Provicol, VOCO GmbH).



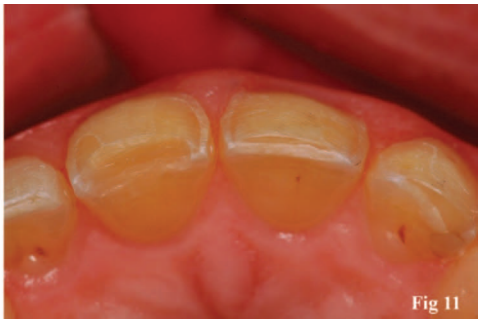


Figure 10. *Incisal grooves.*
Figure 11. *The incisal reduction of the maxillary right central incisor, which was performed in the incisal with a diamond bur at 45° in the direction of palate.*

Lithium disilicate glass-ceramic (IPS e.max Press, Ivoclar Vivadent, Schaan, Liechtenstein) laminate veneers were fabricated with the heatpress technique. They were veneered with a layering ceramic (IPS e.max Ceram, Ivoclar Vivadent) to improve the appearance of the incisal edge. The internal surfaces of the ceramic laminates were treated with 10% hydrofluoric acid for 20 seconds, followed by washing, drying, and application of a silane coupling agent. The tooth surfaces were cleaned with a pumice and prophy cup in a slow speed handpiece and etched with 37% phosphoric acid for 15 seconds, the adhesive system placed (Scotchbond Multi-Purpose, 3M ESPE) and then light cured for 10 seconds. The resin cement, RelyX ARC (3M ESPE), was used. Excess cement was removed with dental floss and dental explorer prior to curing. The resin cement was light cured on the buccal and palatal surfaces of all teeth for 40 seconds. At the end of treatment, the patient was pleased with the esthetics and function of the restorations (Figures 13 through 15). The clinical case was followed up at five years, analyzing the marginal contour, the presence of cracks, staining, care, and patient satisfaction with the restorations (Figures 16 through 18).

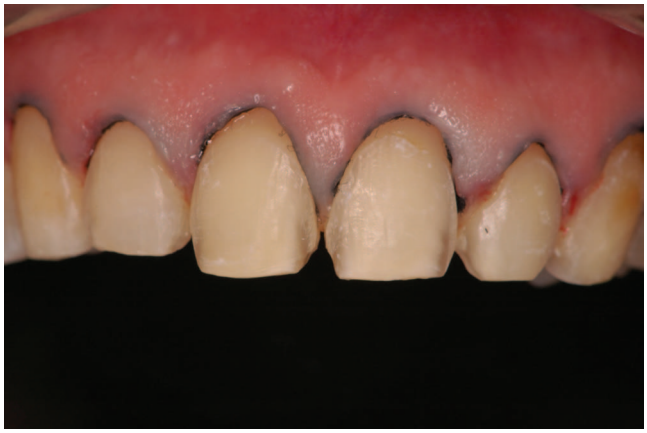


Figure 12. *Finalized and preparations and placement of retraction cord.*

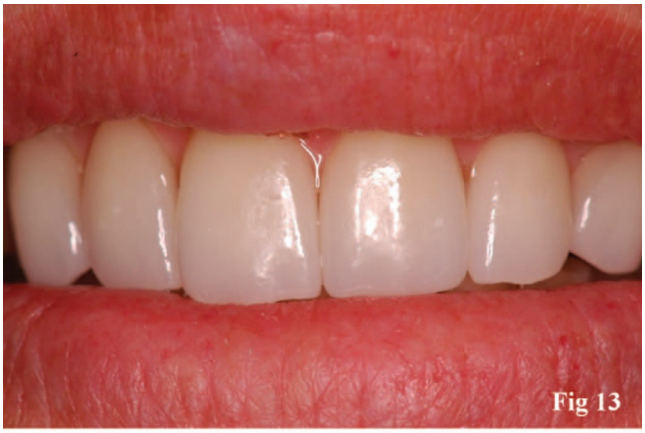
A list of comparable products used in the clinic case that could be substituted in the North American market is as follows: Bis-acryl resin (Structur, VOCO GmbH) can be substituted with Protemp™ (3M ESPE); diamond burs (Jota) can be substituted with diamond burs (SS White Burs, Inc, Lakewood, NJ, USA); and noneugenol temporary cement (Provicol, VOCO GmbH) can be substituted with RelyX™ Temp NE zinc oxide noneugenol temporary cement (3M ESPE).

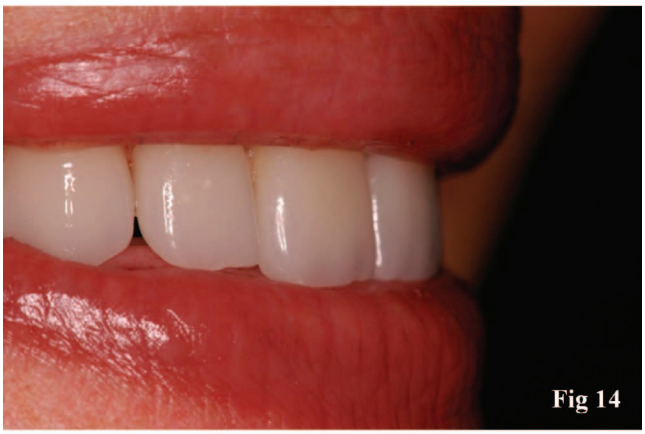
DISCUSSION

When the sequela of tooth erosion, such as loss of tooth structure, are already established, a minimally invasive restorative approach should be chosen, and the etiology should be identified and monitored.[7,9] The diagnosis must be performed with a good history and clinical examination whenever possible, with the full history of general health, diet, evaluation of habits, evaluation of buffering capacity, and determination of salivary flow.[3,10,15] In this current report, a referral to a physician was required to confirm the diagnosis of GERD; rehabilitation was not started until medical treatment controlled the acid reflux.

When there is a need to change the shape, position, and color of dental structures, planning should be done using a diagnostic wax-up.[12] The wax-up will "guide the functional and esthetic procedures," such as aiding in case presentation, demonstrating final veneer form, finalizing incisal-cervical length, estimating thickness of the final veneer, and providing a guide for reestablishing incisal guidance. In this case study, the diagnostic mock-up was essential for the restorative procedures and provided predictable esthetics and maintenance of enamel preparations.

Among the treatment options, ceramic laminates were chosen in this present case because they presented as a conservative option, with the preparation remaining in enamel and in restoring the





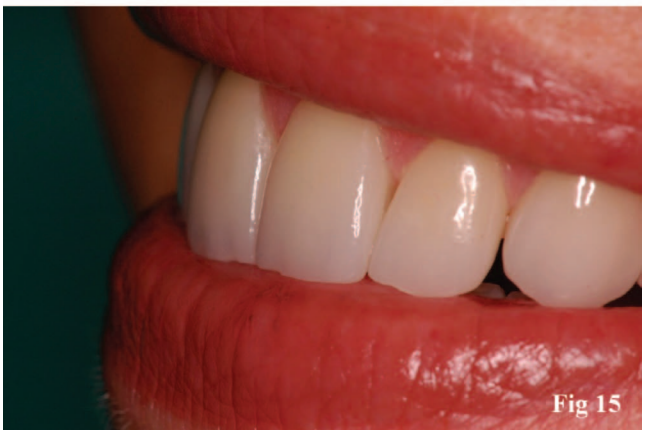


Figure 13. *Appearance of the smile after cementation of the veneers.*
Figure 14. *Right side view of the smile after cementing the veneers.*
Figure 15. *Left side view of the smile after cementing the veneers.*

esthetics and form of the tooth structure. There are numerous advantages and results of ceramic laminates in relation to rehabilitation using composite resins in anterior teeth, including improved color stability, wear resistance, and esthetics.[16] In the present case, a lithium disilicate glass-ceramic was used because of its optical properties and adhesion to

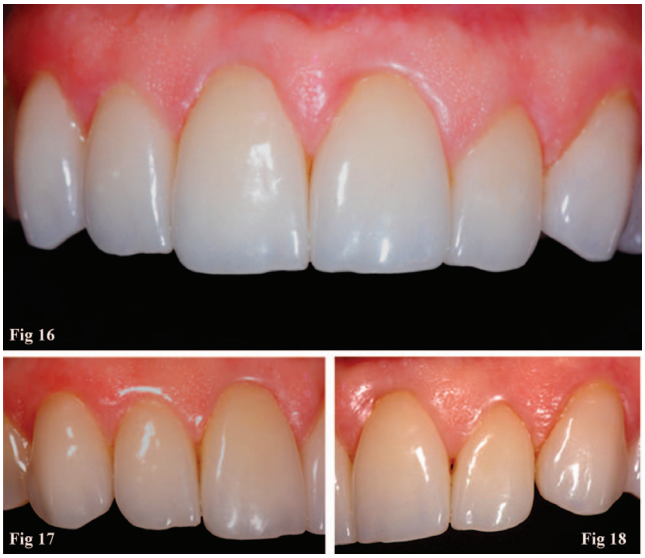


Figure 16. *Appearance of the veneers five years after treatment.*

tooth structure.[17] Also, adequate resistance to support anterior and lateral guidance may have been provided by the low porosity and higher crystallinity of the lithium disilicate glass-ceramic.[18] Because of the relatively low refractive index of leucite and lithium disilicate, even with a relatively high crystalline content, these materials are still sufficiently translucent to restore the incisal edge.[18] Finally, these ceramics are biocompatible restorative materials, which improve the long-term gingival health.[18]

Clinical studies show success, clinical quality, and adequate long-term survival rates of porcelain veneers.[19,20] Given this context, we observed the maintenance of patient satisfaction immediately and after five years of clinical use. The literature reports success rates of ceramic laminates of 98.4% at five years of clinical follow-up[16] and up to 93.5% at 10 years.[21] The technical development of adhesives and composite resins in modern dentistry has provided for clinical predictability of cases with moderate tooth wear. These techniques have enabled the restoration of function and esthetics using more conservative approaches.[9] In this case, the lingual surface was not completely removed to preserve healthy dentin structures because the patient was stabilized in the treatment of gastroesophageal reflux.

Studies claim that predictability and successful outcomes in rehabilitating esthetics depends on correct diagnosis and adequate planning.[1,8,12,19] After four and five years, high patient satisfaction

was observed, the veneers were clinically in good condition, and there was no harm to the gingival health. The only detrimental observations with the current clinical case were staining along the gingival margins, although this had no impact on the clinical success. The definitive success of functional and/or esthetic treatments is only achieved when the patient is well informed and motivated to maintain oral health.[18] Excellent home care and periodic control by the dentist is essential to the long-term success of the rehabilitation.[18]

CONCLUSION

This case demonstrates the success of veneers as an excellent option for effective, conservative, and esthetic treatment for patients with dental erosion, particularly when removing the etiologic factor. It is noteworthy that the noncarious dental lesions caused by acid erosion require an accurate diagnosis for successful rehabilitation.

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.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of Universidade Tuiuti do Paraná.

(Accepted 22 April 2015)

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Clinical Research

Comparison of the Effects of In-office Bleaching Times on Whitening and Tooth Sensitivity: A Single Blind, Randomized Clinical Trial

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Clinical Relevance

This randomized clinical trial provides significant evidence that it is possible to obtain efficacy of whitening with lower tooth sensitivity when in-office bleaching is applied only two times for a period of 15 minutes during each bleaching session.

SUMMARY

Objectives: The objective of the present study was to compare the bleaching efficacy (BE) and tooth sensitivity (TS) of in-office bleaching applied under different time protocols.

Methods and Materials: Fifty-three patients were randomly distributed into three groups: the bleaching agent was applied in one (1×15), two (2×15), or three (3×15) 15-minute applications. The labial surfaces of the anterior teeth were bleached using a 35% hydrogen peroxide gel. Two bleaching sessions with a one-week interval between were performed. The shade evaluation was performed with a visual shade guide and spectrophotometer before and 30 days after bleaching. Participants recorded TS with a five-point verbal scale. Color change was analyzed by one-way analysis of variance and Tukey tests. The absolute risk of TS and TS intensity were evaluated by the Fisher exact and Friedman/Kruskal-Wallis tests, respectively ($\alpha = 0.05$).

Results: Significant whitening was observed in all groups, with statistically lower BE for the

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DOI: 10.2341/15-085-C

1×15 group ($p<0.05$). The absolute risk of TS (95% confidence interval) was lower for the 1×15 group than for the other groups ($p<0.05$). The TS intensity of the 3×15 group was statistically higher than that associated with the other protocols ($p<0.05$).

Conclusions: A single 15-minute application produced less TS but reduced BE. The protocol with 2×15 produced a degree of BE similar to that of the 3×15 group, but with reduced overall TS intensity.

INTRODUCTION

Nowadays, concerns about tooth discoloration have increased[1-3] as more emphasis is being placed on having a "beautiful smile" as an expression of health and vitality, mainly by media and dental manufacturers of tooth-whitening products.[4,5] This has increased the popularity of and demand for dental bleaching.[2,3,6]

Tooth bleaching is the most frequently requested procedure by patients because it is a highly effective and conservative way to improve the appearance of a patient's smile when compared to invasive restorative treatments.[2] Additionally, dental bleaching increases the oral health–related quality of life.[7,8]

In-office dental bleaching has been practiced for more than 100 years and has some advantages over at-home bleaching. In-office bleaching allows close dentist control, avoids material ingestion, and is associated with reduced total treatment time, with great potential for achieving some degree of whitening after one clinical appointment, which enhances patient satisfaction and motivation.[9,10]

However, tooth sensitivity (TS) is a very common adverse effect associated with in-office bleaching.[11-13] Although bleaching-induced TS is not fully understood,[14] it is hypothesized that it comes from the ability of hydrogen peroxide (HP), free radicals, and related by-products to penetrate tooth structure and, upon reaching the pulp,[15,16] produce dental inflammation.[17] At high HP concentrations, the antioxidant capacity of the pulp cells can be easily exceeded, producing oxidative stress and cell damage.[17,18] This explains the higher TS intensity of the in-office bleaching compared to at-home bleaching.[12,13]

In an attempt to reduce this side effect produced by bleaching products, several therapies have been proposed, such as the application of desensitizing agents, administration of analgesics or anti-inflammatory drugs, and the use of bleaching gels containing desensitizing agents[4,9,13,19-25] or the use of in-office bleaching gels with lower HP concentrations.[26,27]

In-office bleaching usually requires a longer application period with changes of the bleaching agent on the tooth surface in each clinical appointment to obtain optimum results.[28-31] In an attempt to reduce the amount of HP that reaches the pulp, researchers investigated whether reduced contact time of the bleaching gels could yield less-adverse effects while still being effective. This approach minimized the deleterious effects of HP to odontoblast-like cells[29-31] and dental pulp of rats[32] while not affecting the overall esthetic outcome in an *in vitro* study.[29-31]

To the best of the authors' knowledge, no clinical study to date has investigated the effectiveness (color change) and side effects (TS) of in-office bleaching performed with a reduced number of changes of the bleaching gel. The null hypotheses tested were that the changes in the in-office bleaching gel would not result in different degrees of 1) color change, 2) absolute risk of TS, or 3) intensity of TS.

METHODS AND MATERIALS

This clinical study was approved by the ethics committee of the local university. The experimental design followed the CONSORT statement.[33] Based on preestablished criteria, 53 volunteers were selected for this study. Two weeks before the bleaching procedures, all of the volunteers received a dental screening and a dental prophylaxis with pumice and water in a rubber cup and signed an informed consent form.

Study Design

This was a randomized, examiner-blind clinical trial with an equal allocation rate. The study took place in the clinics of the Dentistry School of the State University of Ponta Grossa from June 2011 to June 2012.

Inclusion and Exclusion Criteria

Patients included in this clinical trial were men and women between 18 and 30 years of age and had good general and oral health. Participants were recruited from the city of Ponta Grossa (Paraná, Brazil). The participants needed to have six maxillary and mandibular anterior teeth without caries lesions or restorations. The maxillary canine was shade A3 or darker as judged by comparison with a value-oriented shade guide (VITA Classical Shade Guide,

Vita Zahnfabrik, Bad Säckingen, Germany). Participants were excluded from the study if they presented with anterior restorations; had bruxism habits; were pregnant/lactating; were smokers; presented with severe internal tooth discoloration (tetracycline stains, fluorosis, pulpless teeth); were taking any drug with anti-inflammatory, analgesic, or antioxidant effect; or presented with recession and dentin exposure.

Sample Size Calculation

The primary outcome of this study was color change. It had already been reported that two bleaching sessions with the product Whiteness HP Maxx 35% (FGM Dental Products, Joinville, SC, Brazil) produce a whitening effect of around 7 ± 2 shade guide units (SGUs).[9,19,20,26] In order to detect a difference of 2 SGUs between means of any pair of the study groups, with a power of 80% and an alpha of 5%, a minimum sample size of 17 patients was required per group.

Study Intervention

Participants were randomly divided into three groups according to the number of changes of the bleaching gel in each clinical appointment: one 15-minute application (1×15 group); two 15-minute applications (2×15 group), and three 15-minute applications (3×15 group). The randomization process was performed in blocks of three and six using computer-generated tables. This procedure was performed by a third person who was not involved in the intervention procedures. The allocation sequence was placed in sealed and opaque envelopes and was only opened immediately before the beginning of the bleaching protocol.

The participant and the operator could not be blinded to the procedure, as the application of bleaching gel for different times could not be masked. However, the examiners who evaluated the color changes were not aware of which group the participant was assigned to. Before the start of the bleaching procedure, the color was confirmed using an Easyshade spectrophotometer (Vident, Brea, CA, USA), described in detail in the item color evaluation.

Bleaching Procedure

The gingival tissue of the teeth to be bleached was isolated using a light-cured resin dam (Top Dam, FGM Dental Products). In each clinical appointment, the 35% HP Whiteness HP Maxx (FGM Dental Products) was applied in a single (1×15) visit or via two (2×15) or three 15-minute (3×15) applications, following the manufacturer's directions. In the groups in which more than one application was performed, the product on the tooth surface was removed using an aspirating tip, and the product was applied again.

Two bleaching sessions, with one-week intervals between, were performed. All participants were instructed to brush their teeth regularly (four times a day) using fluoridated toothpaste (Sorriso Fresh, Colgate-Palmolive, São Paulo, SP, Brazil) provided by the study investigators.

Table 1: *Color Change Between Baseline and 1-Month Assessment (Means and Standard Deviations) for ΔSGU (Delta Shade Guide Units), ΔL, Δa, Δb, and ΔE for the Three Treatment Groups*[a]

	Groups		
	1 × 15 min	**2 × 15 min**	**3 × 15 min**
Subjective evaluation (ΔSGU)	4.2 ± 1.1 b	7.7 ± 1.3 a	8.0 ± 1.0 a
Objective evaluation (spectrophotometer–CIELab parameter)			
ΔL	0.4 ± 3.3 B	2.4 ± 1.4 A	2.0 ± 1.5 A
Δa	−0.3 ± 0.7 B	−1.5 ± 1.8 A	−1.2 ± 1.4 A
Δb	−5.1 ± 1.8 B	−7.3 ± 3.1 A	−8.3 ± 4.2 A
ΔE	4.5 ± 2.0 B	7.9 ± 2.1 A	8.4 ± 3.6 A

[a] *Comparisons are valid only within rows. Means identified with the same lowercase or uppercase letters are statistically similar (one-way analysis of variance [ANOVA] and Tukey test;* $p < 0.001$).

Color Evaluation

Shade evaluation was recorded before and 30 days after the bleaching treatment using two methods: subjective evaluation using a value-oriented shade guide (Vita Lumin, Vita Zahnfabrik) and an objective evaluation using the Easyshade spectrophotometer (Vident).[22-24]

For the subjective examination, the shade guide's 16 tabs were arranged from highest (B1) to lowest (C4) value. Although this scale is not linear in the truest sense, we treated the changes as representing a continuous and approximately linear ranking for the purpose of analysis. The measurement area for shade matching was the middle third of the facial surface of the anterior central incisor. This measurement was done at baseline and 30 days after bleaching, allowing for the calculation of means and standard deviations of the delta shade guide units (ΔSGUs) of each group.

For calibration purposes, five participants who we did not include in the sample participated in the training phase of this study. The two examiners, blinded to the allocation assignment, scheduled these participants for bleaching and evaluated their teeth against the shade guide at baseline and 30 days after the procedure. The two examiners were required to have an agreement of at least 85% (kappa statistic) before beginning the study evaluation. During the study, if disagreements arose, the examiners reached a consensus before dismissing the patient.

For the objective examination, the color measurement was done with the spectrophotometer Vita Easyshade (Vident). Before the spectrophotometer measurement, an impression of the maxillary arch was taken with dense silicone paste (Coltoflax e Perfil Cub, Vigodent, Rio de Janeiro, Brazil). The impression was extended to the maxillary canine and served as a standard for placement of the spectrophotometer probe in the same place during consecutive color evaluation. The measurement area of interest for shade matching was the middle one-third of the labial surface of the right maxillary canine. A window was created on the labial surface of the molded silicone guide for the central incisor to be evaluated. The window was made using a metallic device with well-formed borders, 3 mm in radius.

The measurement was done on all participants using the Vita Easyshade spectrophotometer (Vident) before and 30 days after the bleaching therapy by only one operator. The shade was determined using the parameters of the Easyshade device which indicated the following values: L*, (a*), and (b*), in which L* represents the value from 0 (black) to 100 (white) and a* and b* represent the shade, where a* is the measurement along the red-green axis and b* is the measurement along the yellow-blue axis. The shade comparison before and after treatment was given by the differences between the two shades (ΔE), which is calculated using the following formula:[22-24] $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$. Three readings were done at each time period, and the shade detected at least twice in these three readings was considered for statistical purposes.

Tooth Sensitivity Assessment

The patients recorded their perception of TS during the first and second bleaching sessions using a five-point rating scale (0 = none, 1 = mild, 2 = moderate, 3 = considerable, and 4 = severe).[9,19] We asked subjects to indicate whether they experienced TS during the treatment and up to 48 hours postbleaching. As two bleaching sessions were performed, the higher score value obtained in both bleaching sessions was considered for statistical purposes. The values were arranged into two categories: overall percentage of patients who reported TS at least once during treatment (absolute risk of TS) and overall TS intensity in different periods (during treatment up to one hour; from one to 24 hours, and from 24 to 48 hours postbleaching). The patients were also instructed to record the painful tooth.

Statistical Analysis

The analysis followed the intention-to-treat protocol and involved all participants who were randomly assigned.[33] The color change (primary outcome) was used to determine the efficacy of the bleaching treatment. The ΔSGU (subjective measurement), ΔL, Δa, Δb, and ΔE (objective measurement) values of different groups were evaluated by one-way analysis of variance (ANOVA). The Tukey test was used for pairwise comparisons.

The absolute risk of TS of both groups was compared using the Fisher exact test. The effect of period (during and up to one hour after procedure; from one to 24 hours after bleaching, from 24 to 48 hours after bleaching) was tested with the Friedman test. The effect of the group on the TS intensity at each period was compared using the Kruskal-Wallis and Mann-Whitney tests. In all statistical tests, the alpha was pre-set at 0.05.

RESULTS

A total of 101 participants were examined to select 53 participants (Figure 1). The mean age (years) of the participants was similar between groups (23.2±3.3, 21.2±4.0, and 25.6±2.4 years, respectively, for groups 1×15, 2×15, and 3×15). The majority of patients were male (58.5%, 72.2%, and 61.1%, respectively, for groups 1×15, 2×15, and 3×15).

Color Change

The mean baseline color of the participants from the three groups was similar between groups (10.4±1.5, 11.5±1.8, and 10.7±1.0, respectively for groups 1×15, 2×15, and 3×15). The subjective and objective evaluations showed a statistically significant higher degree of whitening after one-month postbleaching evaluation for the 2×15 and 3×15 groups compared with the 1×15 group (Table 1; $p<0.001$ for both methods). Whitening of approximately 7.7 and 8.0 SGUs was detected for the 2×15 and 3×15 groups, respectively, and a variation of 7.9 to 8.4 in the ΔE

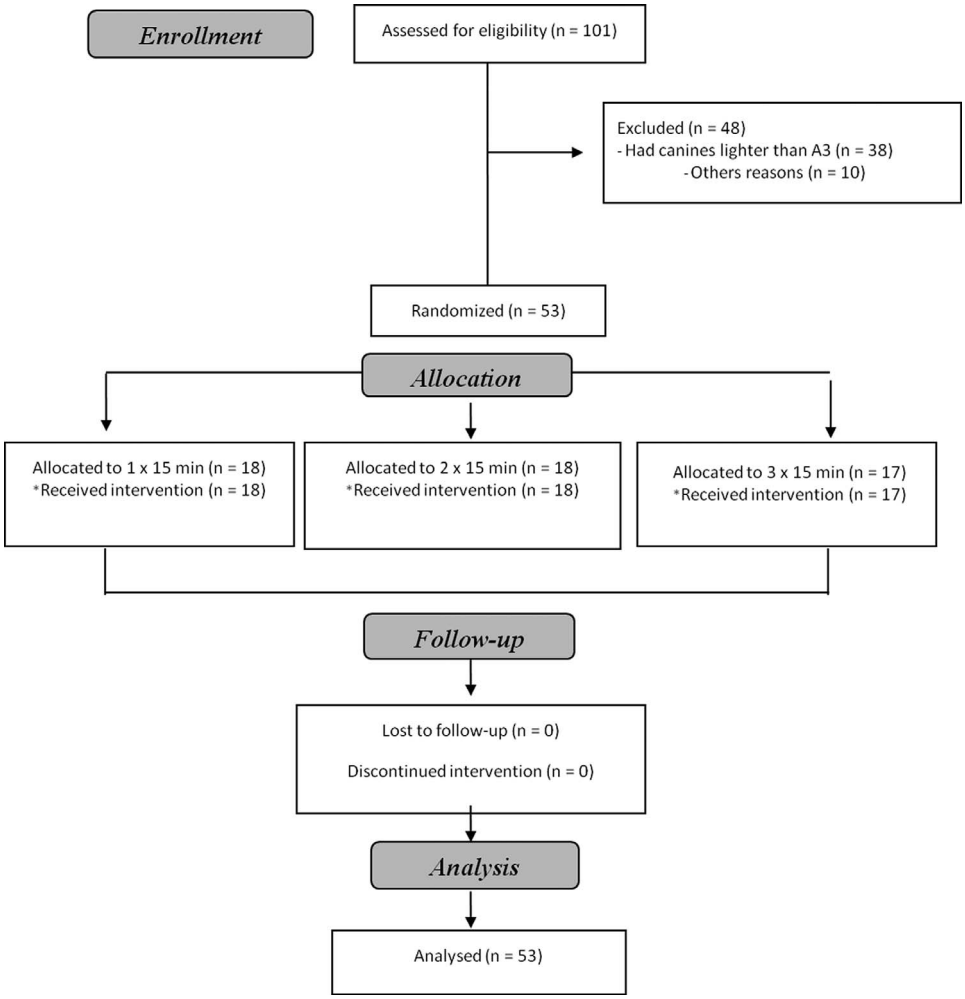

Figure 1. *Flow diagram of the clinical trial including detailed information on the excluded participants.*

was observed for the 2×15 and 3×15 groups, respectively (Table 1; $p>0.32$). On the other hand, only 4.2 SGUs and 4.5 ΔE were observed for the 1×15 group, respectively, in subjective and objective evaluations. The results of the subjective (visual shade guide) and the objective evaluations (spectrophotometer) matched the hypothesis of difference between the groups one month after bleaching ($p<0.001$ for both methods).

Tooth Sensitivity

In regard to the absolute risk of TS, a significant difference was observed between groups (Table 2; $p=0.02$), with the 1×15 group presenting the statistically lowest TS risk. Most of the TS complaints occurred within the first 24 hours after bleaching (Table 3). Only six participants from group 1×15, 9 from group 2×15, and 15 from group 3×15 complained about TS 24 hours after bleaching. The intensity of TS was the highest for the 3×15 group within the first 24 hours (Table 3; $p=0.001$)

DISCUSSION

As a result of the difficulty of measuring color clinically, in the present study we used a shade guide and a spectrophotometer. However, it is also difficult to make a comparison of color change after bleaching, according to previously published literature, because of the different color scales and devices of measurement, as well as the different units of measurement used.[2] Regardless, all in-office bleaching techniques investigated in this randomized clinical trial showed significant color change after two bleaching sessions, which is in agreement with the findings of a recently published literature review[2,34] and clinical trials, mainly for the 2×15 and 3×15 groups after two bleaching sessions,[12,20,24-26,35] which led us not to reject the first null hypothesis.

The whitening effect is related to the concentration, application time, and the number of changes of in-office bleaching gel.[26,29-31,36-38] Despite the find-

Table 2: *Comparison of the Number of Patients Who Experienced Tooth Sensitivity at Least Once During the Three Different Bleaching Regimens Along With Absolute Risks and the Statistical Comparison*[a]

Groups	Tooth Sensitivity, No. of Participants		Absolute Risk (95% Confidence Interval)
	Yes	No	
1 × 15 min	11	07	61 (39-80) A
2 × 15 min	15	03	83 (60-94) AB
3 × 15 min	16	01	94 (73-98) B

[a] *Fisher exact test. Means identified with the same letters are statistically similar ($p<0.05$).*

ings of a recent study[38] that reported that after a single 45-minute application there are still substantial concentrations of HP, this amount may not be enough to sustain the same degree of bleaching obtained in the first 15 minutes, a period during which the availability of HP might be much higher than it is after 45 minutes.[37]

In the present study, there is an association between the effects of application time vs number of gel changes. In the 3×15 group, the gel was maintained for a period that was three times longer, and the gel was refreshed twice more than in group 1×15. The significantly lower amount of gel applied on the tooth surfaces of patients from the 1×15 group can explain the lower whitening effect of this group in comparison to the other groups.[34,39]

Interesting results were found when the bleaching gel was refreshed only twice (2×15), as it reached a similar degree of whitening as was obtained by the conventional 3×15 application suggested for the bleaching gel used. This has an important clinical implication, as it reduces the total chair time and also the amount of bleaching gel required for bleaching, which favors the clinicians' preference for simplification.

Table 3: *Tooth Sensitivity Intensity (Medians and Interquartile Ranges) at the Different Assessment Points for Both Study Groups and the Statistical Comparison*[a]

Assessment Periods	Groups		
	1 × 15 min	2 × 15 min	3 × 15 min
During bleaching up to 1 h	0 (0/1) Aa	1 (0/2) Cb	2 (0/2) Eb
From 1 to 24 h after bleaching	0 (0/1) Ac	0 (0/1) Bc	2 (0/1) Ed
From 24 h to 48 h after bleaching	0 (0/0) Ae	0 (0/0) Be	0 (0/0) De

[a] *Uppercase letters indicate comparisons within groups for the different assessment periods (Friedman test). Lowercase letters indicate comparisons within each assessment period for the different groups (Kruskal-Wallis and Mann-Whitney tests). Similar letters indicate statistically similar medians.*

The absolute risk of TS was greater than 50% in all study groups. The literature reports that TS is a common side effect of bleaching treatments,[11-13,20] and this was confirmed in the present study. HP has a low molecular mass, which favors its rapid diffusion into enamel prisms and interprismatic spaces.[40,41] When it reaches dentin, the HP can easily travel to the pulp chamber through dentinal tubules.[15,16] At high concentrations, HP causes reduction of cell proliferation, metabolism, and viability,[18] and it reduces the pulp-reparative capacity.[42] Additionally, sites of tissue necrosis in teeth with reduced dimensions, such as mandibular incisors, have already been reported.[17] Taken together, these factors may be responsible for the bleaching-induced TS reported by most patients who have undergone bleaching procedures.[11-13,20]

Although a high prevalence of TS was reported in all study groups, the absolute risk and the overall TS intensity were significantly different among them, which led us not to reject the second and third null hypotheses. *In vitro* studies have reported that three consecutive applications of a highly concentrated HP (group 3×15) led to higher amount of HP in simulated pulp chambers,[29-31] with a consequent increase in toxicity to cultured odontoblast-like cells.[29,30] This was much less pronounced with a single 15-minute application (group 1×15).[29,30]

It seems that the bleaching-induced damage of the dental tissue is cumulative and proportional to the amount of HP that reaches this tissue. For instance, Cintra and others[32] evaluated pulp tissue in rats after several bleaching sessions. Significant bleaching-induced changes were observed after one bleaching session with three 15-minute applications, but the extent and intensity of these changes became more severe as more bleaching sessions were performed. Thus, the difference in the amount of the HP that reaches the pulp chamber in the three bleaching protocols might explain the differences in the absolute risk and intensity of TS observed in this study.

Despite the better results in terms of TS in the 1×15 group, this group was not as effective as the others in terms of whitening degree with only two bleaching sessions. However, this does not mean that effective bleaching cannot be achieved. In an *in vitro* protocol, Soares and others[30] demonstrated that five bleaching sessions of a single 15-minute

application can gain the same level of whitening produced by the conventional two bleaching sessions with three 15-minute applications each, with considerably fewer adverse effects. Future clinical studies should attempt to investigate bleaching effectiveness and adverse effects of this shorter protocol of a single 15-minute application for as many bleaching sessions as needed to achieve the patient's satisfaction.

At first glance, multiple bleaching sessions of a single 15-minute application have the problem of increasing the bleaching costs, but this drawback may be outweighed by the reduction in the damage produced in the pulp tissue[29-31] and the reduction in the risk and intensity of TS that arises from this shorter clinical protocol. In addition, a protocol comprising multiple bleaching sessions of a single 15-minute application may play an important role in pulp-dentin stimulation and healing.[43,44]

CONCLUSIONS

Within the limitations of this study, a single 15-minute application of an in-office bleaching gel significantly decreased the risk and intensity of TS but yielded a lower whitening degree after two bleaching sessions. Two 15-minute applications did not reduce the risk of TS but minimized its intensity and whitened to the same extent as did the conventional three 15-minute applications.

Acknowledgements

This study was performed by Carlos Kose in partial fulfillment of his PhD degree at the State University of Ponta Grossa (UEPG), Ponta Grossa, PR, Brazil. The authors would like to thank FGM Dental Products, Brazil, for the generous donation of all products used in this study. This study was partially supported by the National Council for Scientific and Technological Development under grants 301937/2009-5, 301891/2010-9, and 301291/2010-1.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the State University of Ponta Grossa. The approval code for this study is 44/2011.

Conflict of Interest

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

(Accepted 27 May 2015)

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Laboratory Research

Marginal Integrity of Bulk Versus Incremental Fill Class II Composite Restorations

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M El Gezawi

Clinical Relevance

Bulk-fill composites provide similar marginal performance to open-sandwich and incremental composites. *In vitro* use of World Dental Federation criteria can be a valid method for predicting clinical marginal performance of restorations.

SUMMARY

Bulk-fill composites have been introduced to facilitate the placement of deep direct resin composite restorations. This study aimed at analyzing the cervical marginal integrity of bulk-fill vs incremental and open-sandwich class II resin composite restorations after thermomechanical cycling using replica scanning electron microscopy (SEM) and ranking according to the World Dental Federation (FDI) criteria.

Box-only class II cavities were prepared in 91 maxillary premolars with the gingival margin placed 1 mm above and below the cemento-enamel junction. Eighty-four premolars were divided into self-etch and total-etch groups, then subdivided into six restorative subgroups (n=7): 1-Tetric Ceram HB (TC) was used incrementally and in the open-sandwich technique with 2-Tetric EvoFlow (EF) and 3-Smart Dentin Replacement (SD). Bulk-fill restoratives were 4-SonicFill (SF), 5-Tetric N-Ceram Bulk Fill (TN), and 6-Tetric EvoCeram Bulk Fill (TE). In subgroups 1-5, Tetric N-Bond self-etch and Tetric N-Bond total-etch adhesives were used, whereas in subgroup 6, AdheSE self-etch and ExciTE F total etch were used. One more group (n=7) was restored with Filtek P90 Low Shrink Posterior Restorative (P9) only in combination with its self-etch P90 System Adhesive. Materials were manipulated and light cured (20 seconds, 1600 mW/cm^2), and restorations were artificially aged by thermo-occlusal load cycling. Polyvinyl-siloxane impressions were taken and poured with epoxy resin. Resin replicas were examined by SEM (200×) for

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DOI: 10.2341/14-306-L

marginal sealing, and percentages of perfect margins were analyzed. Moreover, samples were examined using loupes (3.5×) and explorers and categorized according to the FDI criteria.

Results were statistically analyzed (SEM by Kruskal-Wallis test and FDI by chi-square test) without significant differences in either the replica SEM groups (p=0.848) or the FDI criteria groups (p>0.05). The best SEM results at the enamel margin were in TC+EF/total-etch and SF/total-etch and at the cementum margins were in SF/total-etch and TE/self-etch, while the worst were in TC/self-etch at both margins. According to FDI criteria, the best was TE/total-etch at the enamel margin, and the poorest was P9/self-etch at the cementum margin.

Groups did not differ significantly, and there was a strong correlation in results between replica SEM and FDI ranking.

INTRODUCTION

Adhesive bonding to tooth structure has been an integral part of modern restorative dental practice that obviously improves the biomechanical and esthetic quality outcomes of restorations.[1,2] An effective bonding to tooth structure would durably seal dentinal tubules and restoration margins, preventing microleakage with adverse consequences of postrestoration hypersensitivity, marginal discoloration, recurrent caries, and harmful effects on the pulp. Furthermore, it would eliminate the need for extension undercuts, thus conserving tooth structure.[3] The literature clearly indicates that all modern formulations of resin adhesives provide an initial satisfactory performance and a progressive gradual *in vivo* deterioration of bonding effectiveness regardless of the bonding approach.[4-6]

Increased patient motivation toward an esthetic, biocompatible, cost-effective, and clinically durable restoration has lead research toward improving the *in vivo* effectiveness and longevity of resin adhesive bonds to tooth structure in direct resin composite restorations, particularly at the cervical margins of class II cavities, where the problem of microleakage becomes more pronounced.[7-9] Clinically effective and durable bonds should resist stresses due to polymerization contraction as well as differences in values of the modulus of elasticity and thermal expansion coefficient between tooth structure and restorative materials. Furthermore, they should be able to survive a number of oral environmental challenges of endogenous collagenolysis, hydrolytic degradation, functional loading, thermal and pH cycling, and bacterial biochemical activities.[3,5,10-12]

Different resin adhesives, placement techniques, and resin composite materials have been suggested to improve the clinical reliability and to control the effect of polymerization contraction stresses. Shrinkage stresses that develop at tooth-restoration interfaces interfere with effective adhesive bonds to tooth structure and marginal sealing of direct composite restorations.[4,5,10-13]

A new generation of bulk-fill resin composites has been recently introduced. Some bulk-fill composites are indicated for use as posterior restorations, while others are used as underlining or base materials under suitable posterior composites. Manufacturers and a few reports indicate that bulk-fill composites provide reduced or relieved interfacial polymerization contraction stresses. Additionally, materials can be applied and light cured in bulk, leading to reduced restorative procedure time, minimized air void entrapment, and improved quality of the final restoration.[2,14,15]

Various *in vitro* tests of marginal adaptation have been widely used to predict the *in vivo* quality of restorations, but reports are inconsistent regarding their clinical relevance. These tests include dye penetration and microscopic marginal and interfacial analysis.[3,4,9,16] While low or moderate correlation was found between scanning electron microscopic (SEM) marginal analysis and clinical findings, no available systematic correlation exists between dye tracing and clinical findings of hypersensitivity, marginal discoloration, caries at restorations margins, and retention.[1] As an *in vitro* test, Heintze[16] suggested using loupes and explorers for the assessment of the marginal integrity of restorations and rating them according to the World Dental Federation (FDI) ranking criteria, which could provide a more clinically relevant testing for marginal integrity.[16,17]

This study was performed in order to comprehensively analyze the cervical marginal behavior of bulk-fill restorations. Replica SEM and simulation of clinical evaluation using FDI criteria were used in an attempt to provide a more clinically relevant study outcome. The null hypothesis was that bulk-fill resin composites provide similar marginal integrity compared to conventional incremental fill and open-sandwich composites. Furthermore, *in vitro*

Table 1: *Materials Used in This Study*

Material	Manufacturer	Lot Number	Description
Tetric Ceram HB	Ivoclar Vivadent	N03283	A light-curing fine-particle microhybrid material based on a moldable ceramic
Tetric EvoFlow	Ivoclar Vivadent	R36640	An incremental light-curing, flowable microhybrid composite
SDR Smart Dentin Replacement	Dentsply	1011002185	A bulk-fill flowable composite base material that allows the curing of layers up to 4 mm thick
SonicFill Composite	Kerr Corp	4252654	Bulk-fill low-shrinkage composite that allows the curing of layers up to 5 mm thick; uses sonic energy during insertion
Tetric N-Ceram Bulk Fill	Ivoclar Vivadent	R65898	Bulk-fill resin composite material that allows the curing of 4-mm-thick layers
Tetric EvoCeram Bulk Fill	Ivoclar Vivadent	R56348	Bulk-fill resin composite material that allows the curing of 4-mm-thick layers
Filtek P90 (Filtek LS), Low Shrink Posterior Restorative	3M ESPE	N 281586	A low-shrink silorane-based resin composite material
Tetric N-Bond Self-Etch	Ivoclar Vivadent	P48222	Light-cured, one-step all-in-one self-adhesive
Tetric N-Bond	Ivoclar Vivadent	R52704	Light-cured primer and adhesive, total-etch adhesive
AdheSE	Ivoclar Vivadent	69346	Two-bottle self-etch adhesive primer and bond
ExciTE F	Ivoclar Vivadent	R50336	Primer and adhesive, total-etch adhesive
P90 System Adhesive, Self-Etch Primer & Bond	3M ESPE	N 281586	Two-bottle self-etch primer and bond
Fine Etch, etchant	Spident	FE1242	37% phosphoric acid gel
Express VPS Impression Material	3M ESPE	N220759	Polyvinylsiloxane impression material

simulation of clinically ranking marginal integrity can provide clinically relevant results.

METHODS AND MATERIALS

A total of 91 human maxillary premolars (n=7/group) that showed no caries, cracks, or developmental defects were used in this study. Teeth were freshly extracted for orthodontic reasons, and their use in research was approved by the local biomedical research ethics committee.

Each premolar was wrapped coronally with wax 2 mm below the cemento-enamel junction (CEJ). An artificial periodontal membrane of about 0.25 mm was created by dipping each premolar once in gum resin (Anti-Rutsch-Lack, Wenko-Wenslaar, Hiden, Germany), followed by lancet trimming of the excess resin apically after resin hardening. Then teeth were embedded vertically in self-curing acrylic blocks to a level 2 mm below the CEJ (self-curing liquid and powder, Major.Ortho, Moncalieri, Italy).

On each premolar, class II mesial and distal box-only cavities were prepared with butt joint margins and 4-mm buccolingual dimensions. Access was gained through enamel with a round tungsten carbide bur (no. 1, HM 1010, Meisinger, Neuss, Germany), and the preparation was completed with a cylindrical diamond abrasive with a flat end (no. 835012, Meisinger); new burs and abrasives were used for each of five cavities. The preparations were performed using high-speed ranges under abundant air-water coolant. The buccolingual dimensions were measured using a digital caliper (Digital Vernier Caliper, Clarke TM International, Essex, UK) with an accuracy of 0.01 mm, the outline was marked with a pencil, and an axial depth of 1.5 mm was measured at the gingival floor using a graduated periodontal probe (1011 Duralite Color Rings, Nordent Manufacturing Inc, Elk Grove Village, IL, USA). In each tooth, the proximal gingival margin was placed 1 mm above the CEJ on one side and 1 mm below it on the other side of the tooth. Consequently, two restorations were inserted in each premolar using the same bonding agent, restorative material, and technique, the only difference being the location of the cervical margin.

According to the resin composite used, 84 specimens were randomly divided into six main study groups and then subdivided according to the bonding technique into self-etch and total-etch subgroups (n=7). Group 7, an additional group (n=7), was included; restored with Filtek P90 low-shrinkage silorane-based composite (3M ESPE, Seefeld, Germany); and bonded with its corresponding self-etch adhesive system. All materials are listed in Table 1 and were manipulated according to the manufacturers' instructions; study variables are displayed in

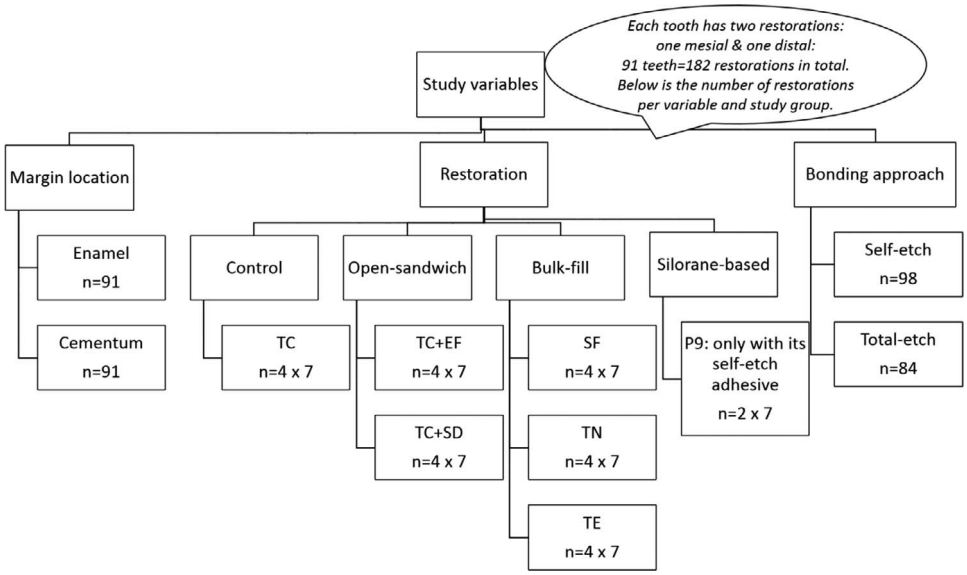

Figure 1. *Overview of the study variables. The abbreviations of the materials are as follows: TC = Tetric Ceram HB, EF = Tetric EvoFlow, SD = Smart Dentin Replacement, SF = SonicFill, TN = Tetric N-Ceram Bulk Fill, TE = Tetric EvoCeram Bulk Fill.*

Figure 1 and the study workflow is displayed in Figure 2.

During the bonding procedure and subsequent application and light curing of the restorative materials, a metallic matrix band attached to a universal matrix retainer (Tofflemire Retainer-Universal, Dentsply, Mount Waverley, VIC, Australia) was used. It was applied to each premolar to maintain the adaptation of the band to the cavity margins. For maximum and rapid curing conversion, the Ortholux Luminous Curing Light (3M Unitek, Monrovia, CA, USA) was used, which is a high-intensity LED of 1600 mW/cm^2 energy output with a wavelength of 430-480 nm and a peak of 455 ± 10 nm. All restorative materials were light cured from an occlusal direction. Each increment of restorative material, conventionally layered or bulk placed, was light cured for 20 seconds, while adhesives were light cured for 10 seconds before composite application, which is in agreement with the manufacturer's instructions. A summary of the various bonding procedures is presented in Table 2. Thereafter, resin composite restorations were inserted according to the assigned study groups.

In group 1, Tetric Ceram HB (TC) resin composite (Ivoclar Vivadent, Schaan, Liechtenstein) was inserted in horizontal increments of 2 mm each by using a metallic plastic instrument (stainless steel, G. Hartzell and Son, San Francisco, CA, USA) and light cured for 20 seconds until complete filling of the cavity. The plastic instrument was used to provide the proper anatomical form before light curing the most superficial increment.

In group 2, the open-sandwich technique was performed by inserting Tetric EvoFlow (EF, Ivoclar Vivadent, Schaan, Liechtenstein) as a base of approximately 2-mm thickness[18] under Tetric Ceram HB (TC+EF), followed by light curing for 20 seconds before incrementally inserting Tetric Ceram HB to completely fill the cavity in a similar manner to group 1.

In group 3, the open-sandwich technique was also utilized by applying SDR Smart Dentin Replacement (SD) bulk-fill flowable resin composite (Dentsply International, Milford, DE, USA) as a base of 4-mm thickness. SD was light cured for 20 seconds before incrementally inserting Tetric Ceram HB (TC+SD) as in the control group TC.

In group 4, SonicFill (SF), a sonic-activated bulk-fill composite system (Kerr Corp, Orange, CA, USA), was inserted into the cavity using the SonicFill Handpiece (Kavo, Biberach, Germany). The SonicFill handpiece automatically dispensed rheologically matched filling materials that are contained in SonicFill Unidose tips into the cavity by the action of sound and pressure under a frequency of 5-6 kHz. A MULTI flex coupling device connected the SonicFill Handpiece to the turbine hose of the dental unit. Initially, SF was inserted in a 5-mm-thick bulk of material and light cured for 20 seconds, and, when needed, a second, thinner horizontal increment was added to complete filling the cavity after reestablishing the occlusal anatomical features.

In group 5, Tetric N-Ceram Bulk Fill (TN; Ivoclar Vivadent) was used. A 4-mm-thick increment was inserted into the cavity using a plastic instrument (G. Hartzell and Son) and light cured for 20 seconds,

Table 2: *Summary of the Bonding Procedure*

Bonding System	Used With	Bonding Procedure
Tetric N-Bond Self-Etch (self-etch)	Tetric Ceram HB incremental Tetric Ceram HB underlined with Tetric EvoFlow Tetric Ceram HB underlined with SDR SonicFill Tetric N-Ceram Bulk Fill	After preparation, cavity was water washed and air-dried; thick layers of Tetric N-Bond Self-Etch were applied to the enamel and dentin surfaces of the preparation and brushed in for 30 s. Excess bonding agent was air thinned and light cured for 10 s.
Tetric N-Bond (total etch)	Tetric Ceram HB incremental Tetric Ceram HB underlined with Tetric EvoFlow Tetric Ceram HB underlined with SDR SonicFill Tetric N-Ceram Bulk Fill	After preparation, cavity was water washed and air-dried, etched for 15 s, and washed with vigorous water spray, and excess moisture was removed. Thick layers of bonding agent were applied to the enamel and dentin using an application brush, air thinned, and light cured for 10 s.
AdheSE (self-etch)	Tetric EvoCeram Bulk Fill	After preparation, cavity was water washed and air-dried. One drop of each primer and adhesive was dispensed individually. Primer was massaged to enamel and dentin for 30 s with a microbrush, air thinned, and light cured for 10 s.
Excite F (total etch)	Tetric EvoCeram Bulk Fill	After preparation, cavity was water washed and air-dried, etched for 15 s, and washed with vigorous water spray, and excess moisture was removed. Thick layers of bonding agent were applied to the enamel and dentin by application brush, and excess adhesive was dispersed with air and light cured for 10 s.
P90 System Adhesive (self-etch)	Filtek P90 (Low Shrink Posterior Restorative System)	Cavity was washed and dried. The self-etch primer was massaged to the surfaces of enamel and dentin for 15 s, air thinned evenly, and light cured for 10 s. Then the adhesive was applied to the entire area of the cavity, air thinned, and light cured for 10 s.

followed by a second increment to completely fill the cavity which was contoured and light cured.

In group 6, Tetric EvoCeram Bulk Fill (TE; Ivoclar Vivadent) was applied in a similar manner to group 5 with different bonding agents: AdheSE (Ivoclar Vivadent), a self-etch adhesive, and Excite F (Ivoclar Vivadent), a total-etch bonding agent, were used in this group.

In group 7, Filtek P90 (P9), a silorane-based composite (3M ESPE), was applied in a similar manner to group 1. P90 System Adhesive (3M ESPE), a self-etch adhesive system, was solely used in this group, according to the recommendations of the manufacturer (Table 2).

After completion of the restorations, gross marginal overhangs were removed by a surgical scalpel blade (no. 15, Swann-Morton, Sheffield, UK). Afterward, restorations were finished and polished using Sof-Lex XT 13-mm-size discs (Sof-Lex XT Finishing and Polishing System, 3M ESPE, St Paul, MN, USA), starting with a courser grit descending down to a superfine grit. The discs, mounted on the Sof-Lex finishing and polishing disc mandrel, were used in a slow-speed range under abundant air-water spray. Following finishing and polishing, the margins of the restorations were carefully inspected using loupes (3.5×) for complete removal of overhangs.

All samples were artificially aged by thermal and occlusal load cycling. Specimens were thermocycled for 5000 cycles in water baths between 5 ± 2°C and 55 ± 2°C at a dwell time of 30 seconds in each bath and a transfer time of 15 seconds between baths (Thermocycling machining, Proto-Tech, El Segundo, CA, USA). Then specimens were subjected to intermittent vertical occlusal loading between 25 and 100 N at 20 cycles/min (20 Hz) for 1000 cycles using the Chewing simulator CS4.2 (SD Mechanotronik GMBH, Westernham, Germany) with a vertically directed round-end piston of 5-mm diameter that touched the occlusal surface at the internal cuspal inclines.

Replica SEM

After artificial aging, polyvinyl-siloxane impressions (Excite, 3M ESPE) were taken of the cervical margins of all restorations mesially and distally. The impression material was injected around the tooth in one direction until the exposed part was completely covered. Impressions were poured with

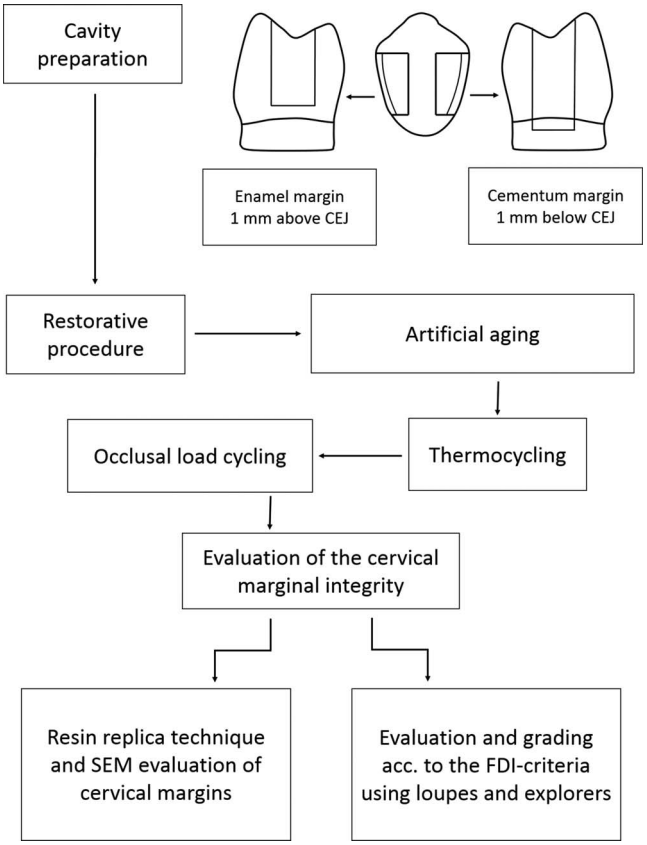

Figure 2. *Work flow of the specimen preparation for replica SEM and FDI ranking.*

an epoxy resin (Epoxy Cure Epoxy System, Buehler, Lake Bluff, IL, USA) for producing resin replicas for the SEM marginal assessment.

After drying at room temperature for 24 hours, replicas were removed from the impressions and trimmed at their cervical bases (Wassermann Trimmer, Hamburg, Germany). Replicas were glued at their bases on a metallic holder and gold sputter coated (25 nm) and examined under SEM at 200× (Stereozoom 250 Microscope, Luxo Microscopes, Elmsford, NY, USA). The quality of the marginal seal at the gingival margins of restorations was categorized into three scores following the methodology of Aschenbrenner and others[19]:

1. Perfect margin: The margin appears with smooth and uninterrupted tooth-restoration continuity.
2. Marginal gap: A distinct gap exists at the tooth-restoration margin.
3. Nonassessable margin: Does not fit the previous two categories accounting for imperfections in the impression material or the epoxy resin.

Images were analyzed with image analysis software (SigmaScan Pro 5.0 image measuring software), and the percentages of perfect margins were recorded.

FDI Ranking

The quality of the margins was assessed with the aid of loupes (3.5×) and two dental explorers with tip diameters of 150 and 250 μm. These explorers were specially prepared for this study by MEDSY dental explorers (MEDSY 560-1, MEDSY, Maniago, Italy). The marginal quality was ranked according to the FDI criteria, as suggested by Hickel and others[17]:

Category 1: Harmonious outline, no gaps, no white marginal lines
Category 2: Small marginal gaps <150 μm indicated by the presence of white lines or small ditching removable by polishing (slight)
Category 3: Marginal gap <250 μm indicated by definite gaps or defects not removable by polishing (major)
Category 4: Gaps >250 μm indicated by base/dentin exposed (severe)
Category 5: Ditching or marginal fracture (larger irregularities)

All samples were evaluated by the same two investigators after conforming to one single reading for each margin. Training on marginal ranking using FDI criteria was performed during the pilot study.

For the SEM results, the statistical analysis was performed with the Shapiro-Wilk confidence test, which proved that values were not normally distributed. Means and standard deviations were calculated, and the statistical analysis was carried out using the Kruskal-Wallis test. For the FDI ranking results, statistical analysis was performed using the chi-square test, while the Spearman correlation coefficient was calculated for correlating the two assessment results (IBM SPSS Statistics 19).

RESULTS

Replica SEM

Figure 3 displays representative SEM images (200×) of the resin replica for each category of margin: perfect margin, marginal gap, and nonassessable margin. The SEM evaluation of the cervical marginal seal was presented quantitatively as mean percentages of perfect margins of all groups in Table 3. None of the test groups showed 100% perfect margins regardless of the test material, bonding technique, or location of the gingival margin.

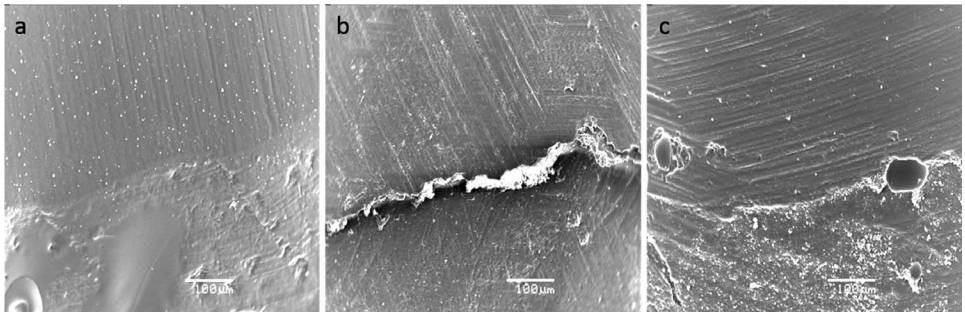

Figure 3. *SEM images (200×) of the resin replica showing a perfect margin (a), a marginal gap (b), and a non-assessable margin (c).*

Statistical analysis revealed no significant difference between the groups (p=0.848). The marginal seal generally trended better at enamel margins than at cementum margins and total-etch groups trended slightly better marginal integrity than self-etch ones, although there was no significant difference between the groups. The values of perfect margins at the enamel sides ranged from 85.6% to 94.9%, while at the cementum sides, they ranged from 70% to 93.3%.

FDI Ranking

The results of the FDI categories in terms of frequencies and percentages for all five categories are presented in Table 4. The chi-square categorical test was applied to compare the groups. The quality of cervical margins ranged from category 1 to category 3 FDI criteria among the study groups. No group consisted completely of category 1 but was rather a mixture of categories. None of the test specimens showed a cervical marginal gap of category 4 or 5.

There was no significant difference between the groups (p>0.05). Enamel margins showed generally better marginal quality than cementum margins. The best marginal quality was found in group TE/total etch at enamel margins. Category 3, the major gap, was found only at cementum margins in TC/self-etch, TC/total etch, TC+EF/self-etch, TC+SD/self-etch, and P9/self-etch. The Spearman rank correlation coefficient (r=−0.632, p=0.001) showed a significantly inverse correlation between the mean percentages of perfect margins of replica SEM and the mean values of FDI ranking (p<0.05; Figure 4).

DISCUSSION

A core issue for a clinically effective and durable resin composite restoration is to maintain tight and leakproof tooth restoration margins.[3,4] Although *in vitro* testing of restorations is an important screening, it does not rule out the clear significance of analyzing the clinical effectiveness of restorations. Lab testing shows different degrees of clinical relevance.[3,16,19] A more clinically relevant testing of marginal sealing necessitates the simulation of the oral environmental factors, including temperature changes, masticatory forces, pH fluctuations, and others.[3,4,16]

A number of studies have listed the pros and cons of different *in vitro* marginal sealing tests, especially toward the validity in predicting the clinical performance of restorations margins.[16] Dye penetration testing is lacking clinical relevance and interstudy comparability. In contrast, the replica SEM method is a well-established procedure that allows for qualitative and quantitative evaluation of margin analysis. Moreover, it can be applied for *in vitro* as well as *in vivo* screening of restorations.[20] Epoxy resin was reported as an adequate material for replicating details of silicone impressions in the indirect study of dentin surfaces.[16,21] An advantage of this qualitative and quantitative method is that

Table 3: *Mean Values of Perfect Margin Percentage (PMP; %) of Replica Scanning Electron Microscopy (SEM) and Standard Deviation (SD)*[a]

Groups	Mean PMP at Enamel	SD	Mean PMP at Cementum	SD
TC/self-etch	85.6	14.9	70.0	21.5
TC/total etch	91.9	11.3	82.3	22.1
TC+EF/self-etch	94.4	14.7	88.7	19.3
TC+EF/total etch	94.9	13.6	88.3	20.1
TC+SD/self-etch	91.4	14.7	79.3	25.9
TC+SD/total etch	93.0	18.5	88.0	20.8
SF/self-etch	93.1	12.4	90.1	17.2
SF/total etch	94.6	9.9	93.3	17.8
TN/self-etch	94.1	15.5	86.0	24.0
TN/total etch	94.1	15.5	81.4	23.5
TE/self-etch	93.7	16.6	91.0	23.8
TE/total etch	92.7	19.3	76.9	29.3
P9/self-etch	93.7	16.6	89.4	28.0

[a] *No significant difference between the groups was observed (p>0.05).*

Table 4: *The Results of the World Dental Federation (FDI) Categories. C1 (Category 1), C2 (Category 2), C3 (Category 3), C4 (Category 4), and C5 (Category 5) Were Presented in Terms of Frequencies and Percentages Between Brackets. The Chi-Square Categorical Test Was Applied to Compare the FDI Ranking Results Between the Groups*[a]

Groups	At Enamel Margins (n=7/Group)					At Cementum Margins (n=7/Group)				
	C1	C2	C3	C4	C5	C1	C2	C3	C4	C5
TC/self-etch	4 (57.1)	3 (42.9)	0 (0)	0 (0)	0 (0)	3 (42.9)	3 (42.9)	1 (14.2)	0 (0)	0 (0)
TC/total etch	5 (71.4)	2 (28.6)	0 (0)	0 (0)	0 (0)	3 (42.9)	3 (42.9)	1 (14.2)	0 (0)	0 (0)
TC+EF/self-etch	5 (71.4)	2 (28.6)	0 (0)	0 (0)	0 (0)	5 (71.4)	1 (14.2)	1 (14.2)	0 (0)	0 (0)
TC+EF/total etch	5 (71.4)	2 (28.6)	0 (0)	0 (0)	0 (0)	4 (57.1)	3 (42.9)	0 (0)	0 (0)	0 (0)
TC+SD/self-etch	5 (71.4)	2 (28.6)	0 (0)	0 (0)	0 (0)	3 (42.9)	2 (28.6)	2 (28.6)	0 (0)	0 (0)
TC+SD/total etch	4 (57.1)	3 (42.9)	0 (0)	0 (0)	0 (0)	5 (71.4)	2 (28.6)	0 (0)	0 (0)	0 (0)
SF/self-etch	4 (57.1)	3 (42.9)	0 (0)	0 (0)	0 (0)	4 (57.1)	3 (42.9)	0 (0)	0 (0)	0 (0)
SF/total etch	5 (71.4)	2 (28.6)	0 (0)	0 (0)	0 (0)	5 (71.4)	2 (28.6)	0 (0)	0 (0)	0 (0)
TN/self-etch	5 (71.4)	2 (28.6)	0 (0)	0 (0)	0 (0)	3 (42.9)	4 (57.1)	0 (0)	0 (0)	0 (0)
TN/total etch	5 (71.4)	2 (28.6)	0 (0)	0 (0)	0 (0)	5 (71.4)	2 (28.6)	0 (0)	0 (0)	0 (0)
TE/self-etch	4 (57.1)	3 (42.9)	0 (0)	0 (0)	0 (0)	3 (42.9)	4 (57.1)	0 (0)	0 (0)	0 (0)
TE/total etch	6 (85.8)	1 (14.2)	0 (0)	0 (0)	0 (0)	3 (42.9)	4 (57.1)	0 (0)	0 (0)	0 (0)
P9/self-etch	5 (71.4)	2 (28.6)	0 (0)	0 (0)	0 (0)	3 (42.9)	2 (28.6)	2 (28.6)	0 (0)	0 (0)

[a] *No significant difference between the groups was observed (*$p > 0.05$*).*

samples can be retested at different study levels, allowing for monitoring restorations over a longer time span.[9,16,19,21,22] Points of weakness in the procedure of the replica technique include the accuracy of the impression relative to the type of bonding agent used, a lack of detailed description of impression taking, and a weak to moderate correlation to clinical findings.[16]

Heintze[16] reported that *in vitro* testing of restoration margins employing a sharp explorer and a magnifying loupe, similar to actual clinical examination, can provide more clinically significant outcomes,[16,17] thus allowing testing at different study stages in addition to saving time and cost.

In the current study, both replica SEM and clinical simulation ranking using the FDI criteria for marginal integrity of restorations were employed in an attempt to provide better prediction for clinical marginal behavior of the test restorations. Our pilot study, as well as previous studies, showed less importance of immediate testing. For this reason, testing was done only after artificial aging by thermoload cycling.[3,4,8,14,22,23]

El-Damanhoury and Platt[2] reported a significant reduction in polymerization shrinkage stress with comparable curing efficiency at 4 mm for some bulk-fill composites, supporting their potential use in posterior teeth. In the current study, a high-intensity LED light-curing unit (1600 mW/cm^2) was used, producing a rapid and high degree of conversion which could lead to greater curing contraction stresses and expose the tested cervical bonded interfaces to increased challenges.[24,25]

Regarding the effect of margin location, the replica SEM results showed that none of the groups could provide 100% perfect enamel or cementum margins regardless of the restorative material or bonding approach used. Although previous studies indicated significantly better marginal integrity at enamel than at nonenamel margins,[22,26] our results found

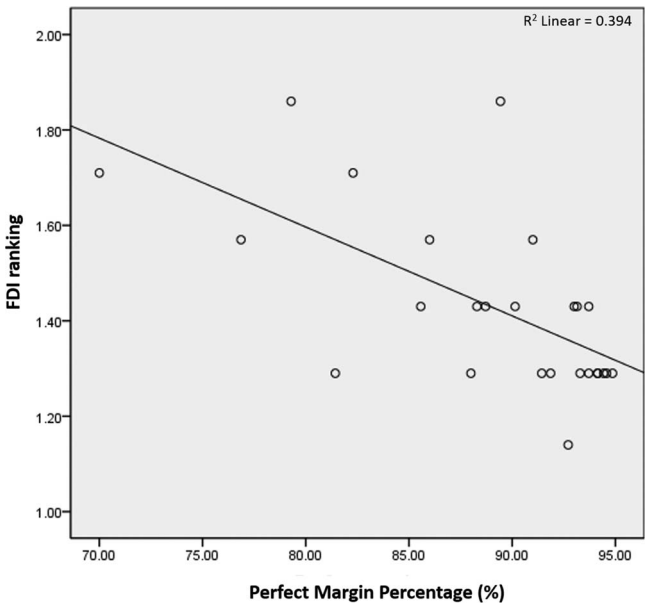

Figure 4. *Graph showing the inverse correlation between the mean perfect margin percentage (PMP; %) of replica SEM and the mean values of FDI ranking (*$p < 0.05$*).*

only insignificantly better marginal integrity at enamel than nonenamel (cementum) margins. This, in part, is in agreement with the findings of Roggendorf and others,[14] who reported different levels of significance in marginal adaptation between enamel and nonenamel margins. They used the replica SEM testing method after thermomechanical cycling of class II composite restorations with and without SD base. In six of their studied groups, enamel margins showed only insignificantly better marginal adaptation than nonenamel margins, two groups showed significantly better adaptation of enamel margins, while two groups showed significantly better adaptation of nonenamel margins. This reflects the apparent impact of the respective restorative material and/or bonding system used on the quality of the outcome.

Because of the increased number of variables and groups of our study and the limited availability of extracted maxillary premolars for orthodontic reasons, the investigators had to decrease the sample size to a low but acceptable sample size (n=7) per group. However, increasing the sample size could have provided a more consistent statistical outcome.

Takahashi and others[22] found a statistically significant higher percentage of continuous enamel margins than dentin margins. Variations in findings can be related to differences in study design and materials. Manhart and Trumm[26] studied the marginal integrity of resin composite restorations in class II cavities after thermoload cycling using the replica SEM. They obtained higher percentages of a "perfect margin" that ranged from 95.9% to 99.6% in enamel and 85.9% to 96.0% in dentin. Sabatini and others[27] studied the effect of preheating and the use of flowable resin liners on the formation of cervical cementum marginal gaps in class II cavities using replica SEM. They found no significant effect on cervical gap formation between their test groups.

There was no clear effect of adhesives in the current study, as the percentages of perfect margin did not differ significantly between the self-etch and total-etch groups. In contrast, Takahashi and others[22] had significantly higher percentages of continuous dentin margins with self-etch than with total-etch adhesives, and an insignificant difference in effect at enamel margins was reported. On the other hand, Roggendorf and others[14] found a significantly higher percentage of continuous margins with total-etch adhesives than with self-etch adhesives at dentin margins with an insignificant difference in effect at enamel margins. Variation in results can be related to materials and testing protocol variations.

The similar marginal sealing quality of bulk and layered resin composites showed in the current study can be explained by previous reports indicating reduced polymerization shrinkage stresses[2] and hence improved marginal behavior of bulk-fill composites.[1,14,15] It is apparent that the initial flowability of SF composite induced by the sonic energy on insertion, as well as the low volumetric shrinkage and high filler loading, compensated for bulk curing by reducing polymerization contraction stresses. This was coupled with adequate resistance to aging by thermoload cycling.[28,29]

Our results showed that using a 2-mm-thick increment of conventional flowable composite did not significantly affect the marginal integrity, differing from Fabianelli and others,[30] who found that using the open-sandwich technique in class II resin composite restorations provided significantly better marginal seal.[30] It was also reported that using an intermediate flowable resin composite with a low modulus of elasticity may partially absorb polymerization contraction stresses in restricted constraints of class II cavities.[23] Differences in restorative materials, flowable liners, bonding systems, and testing procedures may explain variations in results.

Similar to our study, Malstrom and others[18] assessed class II sandwich restorations above and below the CEJ. They found that when margins were located above the CEJ, the use of a 2-mm-thick gingival increment of flowable composite provided significantly less marginal leakage than when 0.5-mm- and 1-mm-thick increments were applied. Nevertheless, de Goes and others[31] suggested that light curing through a thin gingival increment of flowable composite might confirm maximum conversion of the oxygen-inhibited surface molecules of the adhesive layer, thus improving the bond strength. However, this contradicts the common application techniques of composite restorations in class II cavities even with the use of bulk-fill flowable composites.

The present study did not find a significant effect of using silorane-based composite P90 on cervical marginal sealing with either enamel or cementum margins. It was used only with its self-etch adhesive system in order to follow the instructions of the manufacturer as well as other reports.[32,33] On the other hand, Mahmoud and Al-Wakeel[32] found that the use of P90 with its adhesive system significantly improved marginal adaptation compared to Tetric Ceram/Excite in dentin cavities. This was the case immediately after polymerization after one month

and one year of water aging and thermal cycling without occlusal load cycling. Such a variation in effect can be related to the difference in cavity configuration and testing methodology.

Simulating the clinical assessment of marginal adaptation using FDI criteria did not reveal any significant differences between groups, in accordance with results obtained from replica SEM assessment. This is in agreement with the findings of Hayashi and others,[34] who reported that explorer tip diameter had a significant effect on detecting horizontal gaps. In our study, explorer tip diameters of 150 and 250 μm were used according to the recommendation of Hickel and others.[17] Moreover, the inverse correlation between the mean percentages of perfect margins and the mean values of FDI ranking indicates that using loupes and explorers for evaluating marginal sealing by FDI criteria can provide a valid laboratory test for predicting clinical outcomes. Additional research is recommended to correlate *in vitro* results to actual clinical findings using this method. Nevertheless, the results of this study supported the null hypothesis that bulk-fill restoration cervical marginal integrity does not deteriorate and that *in vitro* FDI ranking of marginal sealing can give clinically relevant information on the marginal behavior of restorations.

CONCLUSIONS

Under the circumstances of this investigation, the following conclusions can be drawn:

1. Marginal integrity was not significantly influenced by the use of bulk-fill materials, bonding techniques, or variation in the location of cervical margins.
2. There was an inverse correlation between percentage of perfect margins in replica SEM and marginal ranking according to the FDI criteria, indicating the validity of *in vitro* testing of marginal integrity using this method.

Acknowledgements

The authors would like to thank the University of Dammam, Saudi Arabia, for providing a grant for this research (110/2012). The authors also thank Ivoclar Vivadent, Schaan, Liechtenstein, for supplying the Tetric N-Ceram Bulk Fill, Tetric EvoCeram Bulk Fill, Excite F, and AdheSE materials used in the study.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of: University of Dammam. The approval code for this study is: 110/2012.

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.

(Accepted 22 March 2015)

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Effect of High-Fluoride Dentifrice on Enamel Erosion Adjacent to Restorations *In Vitro*

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Clinical Relevance

Since the prevalence of dental erosion is increasing, use of a high-fluoride dentifrice might be an important strategy to reduce the progression of tooth erosion around restorations.

SUMMARY

Aim: This *in vitro* study analyzed the antierosive potential of a high-fluoride dentifrice on enamel adjacent to restorations.

Methods and Materials: Enamel blocks (6 × 6 × 3 mm) from bovine incisor teeth were restored with three different restorative materials (resin, conventional glass ionomer cement, and resin-modified glass ionomer cement) and treated with dentifrices containing 0, 1100, or 5000 ppm F. After restorative procedures, initial surface Vickers hardness of the blocks were obtained. The specimens were submitted to pH cycles (4 × 90 seconds in soft drink) and treatments for five days. Between the challenges and overnight, the blocks remained in artificial saliva. At the end of the experiment, the final hardness was assessed and the percentage of surface mineral loss (%SML) was calculated. A 3 × 3 factorial design was used to conduct statistical analysis. Data were analyzed by analysis of variance and *t*-test, with significance level fixed at 5%.

Results: High-fluoride dentifrice decreased demineralization caused by erosive challenge regardless of the restorative material used ($p<0.001$). Likewise, the blocks restored with conventional glass ionomer cement showed lower values of SML irrespective of dentifrice used ($p<0.001$).

Conclusion: Use of a high-fluoride dentifrice on teeth restored with conventional glass ionomer cement offers additional protection against enamel erosion.

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DOI: 10.2341/14-292-L

INTRODUCTION

Dental erosion is defined as a chemical process that involves the dissolution of enamel and dentin by

Table 1: *Restorative Materials Used in the Study*

Material	Composition[a]	Manufacturer (Batch)
Filtek Z350 XT (resin)	Organic matrix: BIS-GMA, UDMA, TEGDMA, BIS-EMA, and camphorquinone	3M ESPE. St Paul, MN, USA, LOT 1315600839, Color A2B
	Inorganic matrix: surface-modified zirconia/sílica (3 μm or less), nonagglomerated/nonaggregated 20-nm surface-modified silica particles with 82% by weight (68% by volume)	
Ketac Fil Plus (Conventinal GIC)	Powder: fluoroaluminosilicate glass, strontium, and lantaniumr	3M ESPE, St Paul, MN, USA, LOT 460236, Color A3
	Liquid: polycarbonic, artaric, and maleic acids; wate	
Vitremer (resin-modified GIC)	Powder: fluoroaluminosilicate glass, redox system	3M ESPE, St Paul, MN, USA, LOT 1232700452, Color A3.
	Liquid: aqueous solution of a modified polyalkenoic acid, HEMA	

[a] *Manufacturers' information.*
GIC: glass ionomer cement, BIS-GMA: bisphenol A glycidyl methacrylate, UDMA: urethane dimethacrylate, TEGDMA: triethylene glycol dimethacrylate, BIS-EMA: bisphenol A ethoxylate dimethacrylate, HEMA: 2-hydroxyethyl methacrylate

acids not derived from oral bacteria, leading to a softening of those hard tissues.[1] First, erosive challenge induces enamel surface demineralization by allowing remineralization. Then, a long-term acidic attack causes irreversible loss of hard tissue, which is accompanied by a progressive softening of the surface.[2]

Currently, dental erosion is a frequent condition observed in clinical dentistry.[3] Several methods can be used to prevent or slow the progression of dental erosion, such as dietary intervention, change in consumption of acidic beverages, oral hygiene, and the use of fluoride (F).[4] Regarding F therapies, its effects are reported to be higher when applied at high concentrations, as has already been demonstrated with the use of dentifrices (5000 ppm F), gels (12,300 ppm F), and varnishes (22,500 ppm F).[5-7]

However, the role of F-releasing restorative materials such as glass ionomer cements on the prevention of erosion is not entirely known. It is well established that these cements can release F for a prolonged time and additionally reincorporate it through topical applications, maintaining its preventive effect.[8] Thus, considering that F released from glass ionomer cements can prevent the development of caries adjacent to restorations,[9] it could also reduce the effects of erosion, preventing the defects in dental substrates adjacent to restoration margins.

However, there is a lack of studies showing the combination effect of different F-concentration dentifrices associated with restorative materials on the erosion process. Thereby, the possibility of finding a material or combination of materials that can reduce or prevent dental erosion becomes relevant. Therefore, this study aimed to evaluate the effect of high-F dentifrice in enamel demineralization adjacent to different restorative materials *in vitro*. The null hypotheses tested were that there would not be any effect of type of restorative material or dentifrice use on the response variable assessed.

METHODS AND MATERIALS

Preparation of Blocks

Seventy-two enamel blocks were obtained from the crown of bovine incisors previously sterilized in 10% formol solution, pH 7.0, for at least 10 days. They were flattened and polished by using 400, 600, and 1200 grades of Al_2O_3 papers and polishing cloths with a 1-μm diamond paste, respectively,[10] showing dimensions of approximately 6 × 6 × 3 mm. The specimens were kept moist throughout all of the

Table 2: *Initial Vickers Hardness Number (IVHN), Final Vickers Hardness Number (FVHN), and Percentage of Surface Hardness Loss (%SHL) According to Dentifrices and Restorative Materials Used (Mean± SD, n=8)*[a]

Restorative Material	Dentifrice					
	0			1100		
	IVHN[b]	FVHN[c]	%SHL	IVHN[b]	FVHN[c]	%SHL
Resin	210.40 ± 16.45	124.38 ± 15.34	41.04 ± 3.08	191.13 ± 30.60	126.16 ± 26.72	33.99 ± 9.20
Resin-modified GIC	218.56 ± 19.16	133.09 ± 20.33	39.35 ± 5.00	206.91 ±34.82	151.05 ± 20.22	28.27 ± 8.67
Conventional GIC	224.08 ± 34.59	162.45 ± 34.20	28.05 ± 4.29	202.38 ± 36.90	158.38 ± 31.59	21.35 ± 10.45

[a] p *Values of Analysis of Variance of %SHL data: dentifrice (*$p<0.0001$*), restorative material (*$p<0.0001$*), interaction of dentifrice × restorative material (*$p=0.6416$*).*
[b] *No statistical difference in IVHN among restorative materials groups in each dentifrice treatment (*$p>0.05$*).*
[c] *Statistical difference between IVHN and FVHN among restorative materials groups in each dentifrice treatment (*$p<0.05$*).*

steps. Before restoration placement, all cavities and slab surfaces were cleaned with rotating brushes and non-F dentifrice and washed with distilled water.

Restorative Procedures

Box-shaped standardized cavities (2 × 2 × 2 mm) were prepared at the center of each block with a cylindrical diamond bur (No. 1090, KG Sorensen, Barueri, SP, Brazil) replaced after every 8 preparations. The slots were made with high-speed rotation under water/airspray cooling. Enamel blocks were restored according to the manufacturer's instructions with the following materials: conventional glass ionomer cement (GIC), chemically activated resin-modified GIC, and nanohybrid resin composite as negative control (Table 1).

After placement of the material in the prepared cavity, the surface of the restorative material was covered with a polyester strip and a glass slab under pressure to expel excess material from the cavity. Ionomeric materials were hand mixed and placed in a single bulk with a syringe (Centrix Incorporation, Shelton, CT, USA). In addition, conventional GIC restorations were protected with varnish. For resin-modified GIC restorations, cavities were coated with Vitremer Primer (3M ESPE, St Paul, MN, USA). After primer polymerization, the GIC was placed and polymerized for 40 seconds. For composite resin restorations, cavities were etched for 15 seconds with 35% phosphoric acid (3M ESPE), rinsed thoroughly, and excess water was removed with disposable brushes (Microbrush, Grafton, WI, USA). The cavities were coated with the adhesive system (Single-Bond, 3M ESPE). Composite was placed in three layers of 0.5 mm, each polymerized for 20 seconds with a photo-curing unit. Final restorations were additionally polymerized for 40 seconds.

For all restorations, finishing and polishing were carried out 24 hours later with aluminum oxide discs (SofLex System, 3M ESPE), with each disk applied for 15 seconds. For photo-activated materials, cavities were restored and light polymerized using a halogen-based light-curing unit (Optilux 400, Demetron Research Corporation, Danbury, CT, USA). The light output was tested (480±32 mW/cm^2) before each use with a Demetron Model 100 radiometer (Demetron Research Corporation).

Table 2: Extended.

Dentifrice		
5000		
IVHN[b]	FVHN[c]	%SHL
170.83 ± 36.39	143.56 ± 29.71	15.32 ± 10.44
179.76 ± 53.05	161.93 ± 46.86	9.12 ± 8.50
209.92 ± 36.21	197.00 ± 29.78	5.84 ± 2.92

Dentifrices

Dentifrices were obtained from Manipulation Pharmacy and contained 5000 μg F/g (high concentration of F), 1100 μg F/g (standard concentration), or 0 μg F/g (negative control), all with silica as abrasive, neutral flavor, and F as NaF. Eight blocks restored with each material were randomized for each type of dentifrice studied.

Erosive Challenge

All specimens were submitted to five-day erosion cycles. Erosion was performed with freshly opened bottles of cola soft drink (Coca-Cola, pH 2.6, Brazil; 3 mL/specimen, unstirred, 25°C) four times daily for 90 seconds each. After demineralization, the specimens were rinsed with tap water (five seconds) and transferred into artificial saliva (pH 6.8, 5 mL/specimen, unstirred, 25°C) for one hour. The composition of the artificial saliva was 0.33g KH_2PO_4, 0.34g Na_2HPO_4, 1.27g KCl, 0.16g NaSCN, 0.58g NaCl, 0.17g $CaCl_2$, 0.16g NH_4Cl, 0.2 g urea, 0.03g glucose, and 0.002g ascorbic acid.[11] After the first and last erosive challenge, the blocks were treated with the dentifrice slurries (1:3 dentifrice/water) for one minute. A new aliquot of soft drink was used in each erosive challenge, and artificial saliva was replaced daily. After the last daily erosive treatment, the specimens were stored in artificial saliva overnight.

Determination of Surface Hardness Loss

After restorative procedures, the initial hardness of the enamel blocks was determined 100 μm from the restoration margin, using five indentations, spaced 100 μm from each other, using Vickers microhardness with 100g for 15 seconds. At the end of the experimental phase, the hardness around the restorations was once again measured and reported as a percentage of surface hardness loss (%SHL), using the formula (Initial Hardness – Final Hardness) × 100/Initial Hardness.[12]

Statistical Analysis

A factorial 3 × 3 was considered for the statistical analysis, and the factors under evaluation were the

material used for restoration in three levels (resin, conventional GIC and, resin-modified GIC) and the concentration of dentifrice in three levels (0, 1100, or 5000 ppm F). All data had normal distribution of errors and were analyzed by analysis of variance. The comparison between the initial and final hardness of each group was evaluated by paired *t*-test. The SAS software (version 9, SAS Institute Inc., Cary, NC, USA) was used to perform the statistical tests, with the significance level set at 5%.

RESULTS

The erosive cycling model adopted in this study demonstrated the ability to demineralize the enamel surface, whereas significant differences ($p<0.05$) were observed between the initial hardness and the final hardness (after erosive challenge) for all study groups regardless of the material or dentifrice used (Table 2).

The %SHL data (Table 2) showed significant effects for the isolated factors: material ($p<0.0001$) and dentifrice ($p<0.0001$), but not for the interaction ($p=0.6416$). Thus, lower %SHL was observed in the groups that received the treatment with 5000 ppm F dentifrice, regardless of the material used. Likewise, lower %SHL was observed when the tooth was restored with conventional GIC regardless of the dentifrice used.

DISCUSSION

Treatment for teeth with erosive wear can include minimally invasive therapies for multidisciplinary interventions. Current evidence shows that frequent applications of agents with high F concentration are considered potentially effective approaches to reduce erosive tooth wear.[13] Moreover, in advanced cases of erosion, restorative treatment is necessary; in this case, selection of material depends on its esthetic properties, resistance to biodegradation, adhesive capacity, and F release.[14] Thus, studies evaluating the effect of erosive substances on restorative materials are extremely relevant because most patients have at least one restored tooth and are subject to a contemporary diet containing many erosive substances.[3]

According to the results, mineral loss was found less often in groups treated with the 5000 ppm F dentifrice, regardless of restorative material used. These results agree with those of Moretto and others,[5] who found that an experimental 5000 ppm F dentifrice was able to significantly reduce enamel erosion and erosion abrasion compared with a conventional 1100 ppm F dentifrice *in vitro*. Indeed, high-concentration F applications (such as oral rinses, gels, and varnishes) have been demonstrated to increase abrasion resistance and to decrease the development of erosion in enamel in other studies.[15,16] On the other hand, our findings disagree with those found by Rios and others,[17] because in their *in situ* study, no significant differences were found among 1100 and 5000 ppm F dentifrices for enamel wear. However, it must be considered that a different protocol of treatment with dentifrices was used, because in their study, the exposure to F dentifrice slurry occurred for only 30 seconds, and subjects rinsed their mouths afterward with water.

In addition, regardless of dentifrice used, less mineral loss was found when the tooth was restored with conventional GIC. In fact, lower demineralization closer to GIC restorations was already reported *in situ* and could be explained by a high local F concentration.[18,19] Although the erosive loss of GIC was not evaluated in this study, it has been previously demonstrated that it may be followed by an increase in the pH of the acid solution. This is because of GIC's buffer capacity, which could also protect the tooth from mineral erosive loss.[20]

Although the preventive action of F on dental caries is well documented,[21,22] its role in erosion is still a matter of debate. The present study, despite the limitations of an *in vitro* model, support a positive effect of combination F delivery methods on the enamel demineralization process by either caries or erosive challenge, thereby rejecting the null hypothesis raised.

CONCLUSION

In conclusion, use of a high-F dentifrice on teeth restored with conventional GIC provided an additional protection against enamel erosion; however, *in situ* and clinical trials are required to confirm the relevance of this combination.

Acknowledgment

This study was supported by The National Counsel of Technological and Scientific Development (CNPq, Process: 474318/2013-3), a Brazilian Agency for Research.

Regulatory Statement

This study was conducted in accordance with all the provisions, guidelines and policies of the Federal University of Piauí, Campus Universitário Ministro Petrônio Portella, in Brazil.

Conflict of Interest

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

(Accepted 27 May 2015)

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Effect of Restorative System and Thermal Cycling on the Tooth-Restoration Interface — OCT Evaluation

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Clinical Relevance

The self-etching adhesive system (CSE) showed better dentin marginal integrity after thermal cycling, compared with the etch-and-rinse (SB2), regardless of the type of resin composite used. Enamel was not affected even after thermal cycling.

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DOI: 10.2341/14-344-L

SUMMARY

The present study evaluated the tooth/noncarious cervical lesion restoration interface when using different adhesive systems and resin composites, submitted to thermal cycling (TC), using optical coherence tomography (OCT). Noncarious cervical lesion (NCCL) preparations (0.7 mm depth × 2 mm diameter) were performed on 60 human third molars and randomly divided into six groups, according to the adhesive system and resin composite used: group 1 = Adper Single Bond 2 (SB2) + Aelite LS Posterior (AP); group 2 = SB2 + Venus Diamond (VD); group = SB2 + Filtek Z250XT (Z250); group 4 = Clearfil SE Bond (CSE) + AP; group 5 = CSE + VD; group 6 = CSE + Z250. Selective enamel etching was performed for 30 seconds on groups 4, 5, and 6, while groups 1, 2, and 3 were etched for 30 seconds in enamel and 15 seconds in dentin. All groups were evaluated using OCT before and after TC (n=10). Images were analyzed using Image J software; enamel and dentin margins were separately

evaluated. Data from OCT were submitted to PROC MIXED for repeated measurements and Tukey Kramer test ($\alpha = 0.05$). No marginal gaps were observed in etched enamel, either before or after TC, for all adhesive and resin composite systems. A significant interaction was found between adhesive system and TC for the dentin groups; after TC, restorations with CSE showed smaller gaps at the dentin/restoration interface compared with SB2 for all resin composites. Increased gap percentages were noticed after TC compared with the gaps before TC for all groups. In conclusion, TC affected marginal integrity only in dentin margins, whereas etched enamel margins remained stable even after TC. Dentin margins restored with CSE adhesive system showed better marginal adaptation than those restored with SB2. Resin composites did not influence marginal integrity of NCCL restorations.

INTRODUCTION

Clinical success of resin composite restorations is fundamentally dependent on effective and durable bonds to enamel and dentin.[1] Marginal sealing is one of the most important factors influencing the success of a restoration.[2]

Although an intimate bond is extremely important, a perfect margin is difficult to achieve[3] because of intrinsic characteristics of the materials. Gaps can occur in enamel and dentin because of loss of internal adaptation among dental hard tissues and the resin composite material. Additionally, bonding to dentin is the most difficult type of bond to achieve.[4]

With regards to clinical success, one of the most important factors to be considered is composite polymerization shrinkage.[5] Over the past few years, manufacturers have invested in the development of low-shrinkage resin composites. They claim that such materials produce a lower percentage of shrinkage compared with conventional composites, which would be extremely useful in improving the marginal adaptation of restorations.[6] Shrinkage from polymerization can result in marginal gaps and leakage, tooth fracture, composite fracture, dislodgement of the restoration, and postoperative sensitivity.[2] Thus, the use of a resin composite with an appropriate elastic modulus and low rate of polymerization shrinkage, combined with an adequate dentin adhesive system, could be an effective way to restore cervical lesions.[7]

Because of the unique characteristics of noncarious cervical lesions (NCCLs)—such as a sharp wedge-like morphology; a frequently subgingival location,[8] which includes enamel and dentin margins; and the need for supporting occlusal and brushing forces—the adhesion of materials to these lesions becomes a challenge. NCCL Class V cavities are frequently used to clinically evaluate the effectiveness of adhesive systems.[9] High adhesion levels are necessary to fulfill such tasks as sealing dentinal tubules to reduce postoperative sensitivity, sealing restoration margins to reduce the risk of marginal staining and marginal caries, and keeping the restoration in place.[9] In the case of retention, while studies with class I and II cavities are of great value[10,11] when evaluating a restoration, the preparation and/or caries removal normally generates adequate mechanical retention, thus making adhesion to tooth substance less important.[9] Adhesion to tooth substrate is more necessary when there is not sufficient retention, as with NCCL restorations.[9]

Regarding the conventional bonding technique, etching dentin is an aggressive procedure, as it dissolves and removes the natural collagen protection, thereby producing a resin-collagen complex that is vulnerable to degradation by water sorption, which is possibly enhanced by the documented enzymatic degradation process.[12] The advent of two-step self-etching adhesive systems introduced a new perspective, as these materials limit dentin mineral dissolution while simultaneously replacing minerals with resin monomers.[13] Although self-etching adhesive systems exhibit the best dentin marginal quality, they frequently do not show the same superiority in enamel, as demonstrated by Frankenberger and others.[14] When using this type of adhesive, prior selective enamel etching is commonly indicated to promote a superior demineralization and micromechanical retention.[14,15] Even so, the strongest chemical bonds can be weakened when subjected to repeated disruptive stresses in the oral environment.[8]

In vitro studies can be performed to simulate oral environment stresses. Thermal cycling (TC) simulates temperature changes in the oral environment.[16] TC effects are deleterious to the tooth/restoration bonding interface and can accelerate exposure of resin components to hydrolytic degradation or significant temperature oscillations, which may cause tensions in the bonding interface.[17]

Evaluation of the tooth/restoration interface may be performed by various methods. In an attempt to elucidate problems related to destructive analyses,

Table 1: *Composition, Manufacturers, Batch Numbers, and Protocol for Applying the Materials Studied*

Material (Group)	Manufacturer (Batch no.)	Composition	Application Protocol
Clearfil SE Bond (CSE)	Kuraray Inc, Osaka, Japan (Primer: 01108A; Bond: 01657A)	Primer: HEMA (10-30 wt%), MDP, hydrophilic aliphatic dimethacrylate, dl-camphorquinone, water, accelerators, eyes, others Bond: BISGMA (25-45 wt%), HEMA (20-40 wt%), MDP, hydrophobic aliphatic methacrylate, colloidal silica, dl-camphorquinone, initiators, accelerators, others	Etch with 35% phosphoric acid on enamel for 30 s. Rinse for 30 s. Blot excess water using a humid cotton pellet. Immediately after blotting, apply a coat of the primer with gentle agitation and using a fully saturated applicator for 20 s. Gently air thin and apply a coat of the bond. Gently air thin for 5 s to evaporate solvent. Photoactivate for 20 s.
Adper Single Bond 2 (SB2)	3M ESPE Dental Products, Sumaré, Brazil (N3025G0BR)	Ethyl alcohol (25-30 wt%), silane-treated sílica (nanofiller) (10-20 wt%), BISGMA (10-20 wt%), HEMA (5-15 wt%), glycerol 1,3-dimethacrylate (5-10 wt%), copolymer of acrylic and itaconic acids (5-10 wt%), UDMA (1-5 wt%), water (<5 wt%)	Etch with 35% phosphoric acid, applied first to enamel (30 s) and then to dentin (15 s). Rinse for 30 s. Blot excess water using a humid cotton pellet. Immediately after blotting, apply a coat of adhesive with gentle agitation using a fully saturated applicator for 20 s. Repeat the application. Gently air thin for 5 s to evaporate solvent. Photoactivate for 20 s.
Aelite LS Posterior (AP)	Bisco, Schaumburg, IL, USA (111200007310)	Bis-EMA (<25 wt%), glass filler (<65 wt%), amorphous silica (<15 wt%)	Place in a single increment; photoactivate for 20 s
Venus Diamond (VD)	Heraeus Kulzer Inc, Hanau, Germany (010039)	TCD-DI-HEA, UDMA, barium aluminum fluoride glass, highly discrete nanoparticles (contains 64% filler by volume, 5 nm-20 μm)	Place in a single increment; photoactivate for 20 s
Filtek Z250 XT (Z250)	3M ESPE, Sumaré, Brazil (N333058BR)	Silane-treated ceramic (65-90 wt%), BISGMA (1-10 wt%), Bis-EMA (1-10 wt%), silane-treated silica (1-10 wt%), UDMA (1-10 wt%)	Place in a single increment; photoactivate for 20 s

nondestructive methods used to evaluate marginal integrity of restorations have been studied. Recently, there has been an increasing interest in technologies that can reconstruct images of the internal structures to study defects of resin composites, adhesion, and shrinkage phenomena with a minimum of technique artifacts.[18] Optical coherence tomography (OCT) is an emerging technology that has demonstrated its utility in assessing and visualizing internal biological structures and some biomaterials in a noninvasive and nondestructive manner.[10,11,18-21] OCT has been used to identify and quantify marginal gaps under a resin composite restoration without specimen cross-sectioning.[10,11,18,19,21] In a recent study, Bakhsh and others,[11] showed that OCT images of some cavities provided increased signal intensity that appeared as bright clusters at the cavity floor and represented a gap at the bonded interface.

Thus, the aim of this study was to use OCT to quantitatively evaluate how different adhesive systems associated with resin composites of different shrinkage rates as well as TC affect NCCL marginal integrity (enamel/dentin). The following hypotheses were tested: 1) TC affects marginal integrity of the enamel-dentin/restoration interfaces; 2) the self-etching adhesive system produces a lower marginal gap percentage in enamel and dentin compared with the etch-and-rinse adhesive system; and 3) low shrinkage resin composites produce a lower marginal gap than conventional resin composites.

METHODS AND MATERIALS

Restoration Procedures

The materials, manufacturers, composition, batch number, and application protocol of the materials used in this study are indicated in Table 1.

Sixty sound, freshly extracted, human third molars were obtained according to protocols approved by the ethical committee and stored in deionized water at 4°C for up to 30 days. A standardized NCCL preparation (0.7 mm depth × 2

mm diameter) was performed in each tooth, with cavity margins located in both enamel and dentin. Preparations were created using one diamond bur (standard grain 75-125 μm, no. 3131, Microdont, São Paulo, Brazil) for every five cavities in a high-speed handpiece with a cooled water spray using a standardized cavity preparation machine.[22] The teeth were pumiced and then randomly divided into six groups (n=10) according to the adhesive system and resin composite used: group 1 = Adper Single Bond 2 (SB2) + Aelite Posterior (AP); group = SB2 + Venus Diamond (VD); group 3 = SB2 + Filtek Z250 XT (Z250); group 4 = Clearfil SE Bond (CSE) + AP; group 5 = CSE + VD; and group 6 = CSE + Z250. Bonding and restorative procedures are indicated in Table 1. Enamel etching was performed for 30 seconds before application of the adhesive systems for all groups, while dentin etching was performed for 15 seconds only for the groups with the SB2 adhesive system.[14] Composites were photo activated according to the manufacturer's recommendations, using a light-emitting diode curing light (700 mW/cm^2 intensity; Elipar Freelight 2, 3M ESPE, St Paul, MN, USA). Teeth were then stored for 24 hours at 37°C at 100% humidity. Finishing and polishing were performed using a sequence of medium, fine, and superfine aluminum-oxide abrasive disks (Sof-Lex Pop On, 3M ESPE) for 15 seconds each.

OCT Evaluation

After preparation and polishing procedures, a silicon specimen holder (Silon 2APS, Dentsply, Catanduva, Brazil) was fabricated for each specimen to individually fix it to the OCT worktable (OCS1300SS, Thorlabs Inc, Newton, NJ, USA) and allow identical assessment of each specimen before (baseline) and after TC. Images were obtained by scanning the buccal surface in the mesiodistal direction over the restoration. Five images were obtained every 0.33 mm.

Marginal Gap Percentage Calculation

Images were quantitatively analyzed using Image J software (Image J 1.45, NIH, Bethesda, USA).[23]

Enamel Marginal Gap Percentage Calculation

First, total enamel marginal length was calculated. The enamel marginal gap was linearly measured along the enamel margin. Then, a percentage of enamel marginal gap was calculated using equation 1, where %G1= % enamel marginal gap at baseline; L_e= total enamel marginal length; and l_e= enamel marginal gap length:

Equation 1: %G1 = (le / Le) *100

After baseline evaluation, specimens were thermal cycled for 1000 cycles (30 seconds in each bath of 5°C and 55°C water, with an interval of 30 seconds in a 37°C bath) in a thermal cycling simulator machine (MSCT-3, Elquip, São Carlos, Brazil). After TC, each specimen was fixed on the holder and the gap evaluation was carried out again, using the same parameters and locations as the baseline, to obtain %G2:

Equation 2: %G2 = (le / Le) *100

The enamel marginal gap was calculated as follows: $\%Gap = \%G2 - \%G1$, where %G1 = the pre-TC enamel marginal gap and %G2 = the post-TC enamel marginal gap.

The same procedures for enamel marginal gap measurement were conducted to measure dentin marginal gap, with the dentin marginal gap percentage calculated as follows:

1. Dentin gap percentage at baseline: $\%G1 = (ld/Ld) \times 100$
2. Dentin gap percentage after TC: $\%G2 = (ld/Ld) \times 100$
3. Dentin gap percentage: $\%Gap = \%G2 - \%G1$

Only the image with the highest percentage of enamel and dentin marginal gaps from each group was considered for statistical analysis.

After exploratory data analysis of enamel and dentin gap percentages, as analyzed by OCT, the methodology of mixed models for repeated measures (PROC MIXED for repeated measures) and the Tukey Kramer test for comparison between groups were applied. Level of significance was set at 5%.

RESULTS

No gaps were observed at the enamel/restoration interface before or after TC for any resin composite or adhesive system used (Figures 1 through 3). When considering the dentin/restoration interface, statistical analysis showed no interaction among the three factors studied (resin composites, adhesive systems, and TC) (p=0.3557). However, there was a significant interaction between the factors of adhesive system and TC (p<0.0001) (Table 2).

Table 2 shows the results of the OCT analysis of the dentin/restoration interface, providing the mean percentage and standard deviation of the dentin gap formation based on TC, resin composite, and adhesive system. The CSE and SB2 adhesive systems showed significantly higher dentin marginal gap

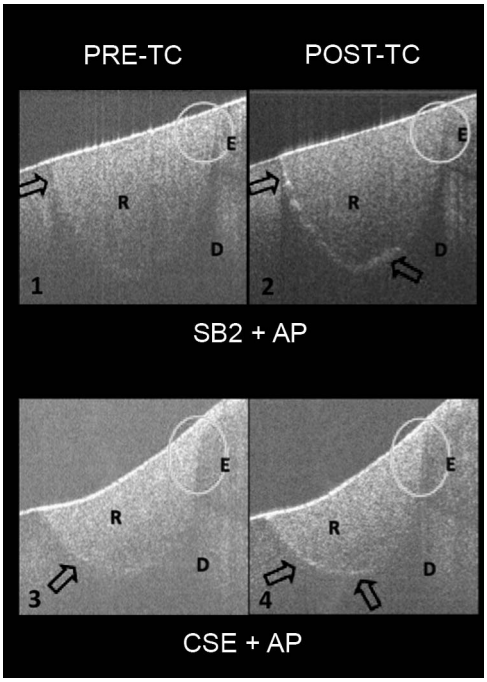

Figure 1. *OCT image from a sample restored with AP resin composite. The first column shows OCT images before TC, and the second column represents after TC. The first line shows samples restored with the SB2 adhesive system, and the second line shows samples restored with CSE. Arrows indicate gaps in the resin/dentin interface observed by OCT. Note the absence of resin/enamel interfacial gaps, even after TC, shown in circles. R, resin composite; E, enamel; D, dentin.*

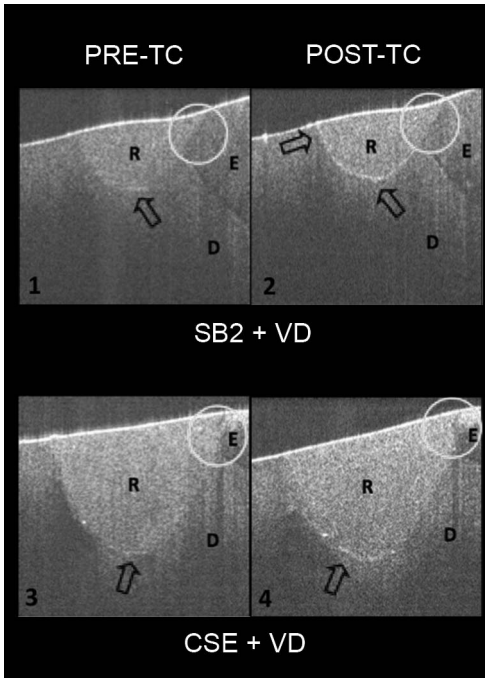

Figure 2. *OCT image from a sample restored with VD resin composite. The first column shows OCT images before TC, and the second column represents after TC. The first line shows samples restored with the SB2 adhesive system, and the second line shows samples restored with CSE. Arrows indicate gaps in the resin/dentin interface observed by OCT. Note the absence of resin/enamel interfacial gaps, even after TC, shown in circles. R, resin composite; E, enamel; D, dentin.*

means at the resin/dentin interface after TC than the same groups at baseline.

When analyzing the effect of the adhesive systems after TC, restorations using SB2 showed a higher mean percentage of dentin marginal gaps, compared with those obtained with CSE, for all tested resin composites. No significant difference in marginal gaps was found between measures obtained before and after TC for all resin composites used. Figures 1 through 3 show OCT images before and after TC for each resin composite and adhesive system in the enamel and dentin margins.

DISCUSSION

The first hypothesis was partially accepted because TC did not affect enamel/restoration margins but did affect dentin/restoration margins.

An absence of enamel marginal gaps was observed in all groups, even after TC. These results were different from those obtained by Makishi and others,[10] who used 5000 cycles. Those authors observed an increase in enamel gap formation after TC. The difference in these results demonstrates that long-term TC can present differences in the bonding of different adhesive systems to enamel. Although ISO TR 11450[24] recommends 500 cycles as a methodology for aging studies, the current study used 1000 thermal cycles and still did not demonstrate differences between groups. In addition, this difference in results might be attributed to the enamel surface acid etching before the use of the self-etching adhesive system, which was not performed in the study by Makishi and others.[10] Although all self-etching adhesives bond reasonably well to ground enamel, there is a general consensus that the milder versions of these adhesives do not etch well on unground surfaces, where there is no resin tag formation and little subsurface demineralization for micromechanical retention.[15,25,26] Thus, we chose to do enamel etching using phosphoric acid before CSE application, as indicated by Frankenberger and others[14] It can improve the bond stability of these adhesive systems to enamel, hindering the formation of marginal gaps and producing greater longevity for the restoration.[14] This may have contributed to the absence of statistical difference between adhesive systems in enamel margins.

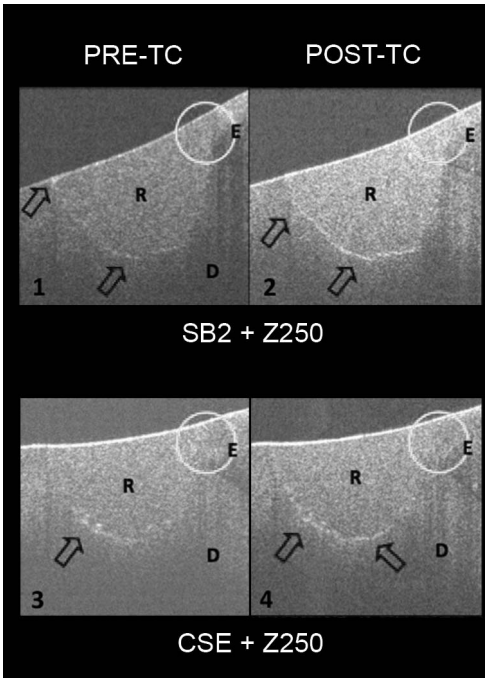

Figure 3. *OCT image from a sample restored with Z250 resin composite. The first column shows OCT images before TC, and the second column represents after TC. The first line shows samples restored with the SB2 adhesive system, and the second line shows samples restored with CSE. Arrows indicate gaps in the resin/dentin interface observed by OCT. Note the absence of resin/enamel interfacial gaps, even after TC, showed in circles. R, resin composite; E, enamel; D, dentin.*

In this study, enamel etching was previously performed for both adhesive systems, and may have improved the micromechanical interlocking of resin tags in the conditioned enamel surface.[14,27] This procedure not only seals the restoration margins in the long term but also protects the dentin bond, which is more vulnerable to degradation.[28]

Images from OCT allowed differentiation between enamel, dentin, gaps, and resin composite (Figures 1 through 3). Clinically, OCT real-time visualization of tissue microstructure can prevent patients from the need to remove tissues and process specimens as well as from exposure to a radiation dose. OCT images differentiate the tissue optical properties, which include the effects of optical absorption and scattering.[29] Even carious demineralization can be seen in OCT through noninvasive and instant images.[20] Bonding to dentin has been a challenge when considering bond durability, as this substrate has significant structural and morphologic heterogeneity compared with enamel.[14,27,30]

The second hypothesis was partially accepted because there was a significant difference between the adhesive systems when bonded to dentin. The

Table 2. *Mean Percentages and Standard Deviations of the Variation of Gaps at the Dentin/Restoration Interface Based on Thermal Cycling, Composite Resin, and Adhesive System*[a]

TC	Composite	Adhesive System	
		CSE	SB2
Before	AP	*4.20 ± 2.06 Aa	*4.90 ± 3.50 Aa
	VD	*6.05 ± 4.45 Aa	*7.70 ± 3.59 Aa
	Z250	*6.87 ± 2.97 Aa	*7.18 ± 3.05 Aa
After	AP	6.11 ± 2.65 Ba	11.34 ± 7.5 Aa
	VD	8.49 ± 5.90 Ba	17.51 ± 7.39 Aa
	Z250	10.26 ± 4.75 Ba	16.29 ± 6.73 Aa

[a] *Means followed by different letters (uppercase in horizontal and lowercase in vertical) differ from each other ($p \leq 0.05$) within the same group of cycling. * Differs from the mean after cycling in the same composite and adhesive system ($p \leq 0.05$).*

SB2 adhesive system showed greater gap percentages in dentin after TC compared with CSE, for all composites evaluated.

These results are in accordance with Frankenberger and others,[13] who observed that dentin bonding systems with a separate hydrophobic component, such as CSE, are less sensitive to thermomechanical cycling. Furthermore, the additional ionic bonding with residual hydroxyapatite is reached from etched dentin when a mild self-etching adhesive system is used.[27]

The better performance of CSE in dentin can be attributed to the presence of a 10-methacryloyloxydecyl dihydrogen phosphate (MDP) monomer in its composition. According to Nurrohman and others,[31] when CSE is used, hydroxyapatite crystals remain within the hybrid layer. The residual hydroxyapatite crystals can serve as a template for the chemical reaction with functional groups such as MDP.[32] In the chemical interaction of hydroxyapatite with the adhesive components, MDP bonds strongly to crystal, forming a stable link between calcium and MDP.[32,33] Therefore, MDP is a monomer that has hydroxyapatite affinity with chemical functional groups. After polymerization, monomers individually bond to the tooth's hydroxyapatite and form a polymer.[34]

When SB2 was used, a higher percentage of post-TC gaps can be attributed to dentin etching, which seems to dissolve and remove the natural protection of collagen, making the complex collagen-resin more vulnerable to degradation by water sorption, which is possibly increased by the process of thermal degradation during TC.[35] These two simultaneous stresses (water immersion and temperature chang-

es) can destabilize the bond between adhesive system/tooth[27] and promote the hydrolytic degradation of resin components in the adhesive interface.[17]

Clinically, the etch-and-rinse technique leads to a question concerning the wettability of the dentin. It is not uncommon to have overly wet regions and overly dry surfaces in the same preparation, which causes a nonuniform resin bonding. Etch-and-rinse adhesive systems are more technique sensitive because optimal hybridization and sealing of dentinal tubules with the wet bonding technique may differ with each bonding system.[36] Although most bonded restorations are retained because there is sufficient well-bonded surface area, a common clinical manifestation of inconsistent bonding within a restoration is the patient's complaint of postoperative sensitivity.[37-39] Self-etching adhesives can surpass these problems because dentin must not be etched. It reduces postoperative sensitivity due to the decrease in hydraulic conductance through the dentinal tubules.[40,41]

The third hypothesis was rejected because none of the resin composite group comparisons showed a significant difference, either pre- or post-TC degradation.

This finding is in agreement with Baracco and others,[42] who compared *in vivo* restorations made with conventional and low-shrinkage resin composites. Those authors showed declining marginal adaptation scores in the restorations placed with all systems and concluded that the low-shrinkage resin composite used in their study provides adequate clinical performance but does not surpass the behavior of methacrylate-based conventional materials. On the other hand, Yamamoto and others[43] compared the polymerization stress of low-shrinkage and conventional resin composites, observing that the VD resin composite showed lower polymerization stress values compared with a conventional dimethacrylate resin composite. Finally, those authors concluded that, because of the many factors that influence polymerization stress development in resin composites, reduced shrinkage itself does not always generate lower stress[43] or higher levels of restoration marginal integrity.[42]

The results obtained in our study can be related to the depth of the cavity used. According to Braga and others,[44] there is a direct relationship between stress generated by polymerization shrinkage and the depth of a cavity. Those authors concluded that the volume shrinkage of the composite doubles as the depth of the cavity increases from 1 to 2 mm. Therefore, it is assumed that deeper cavities have higher shrinkage stress and, consequently, more marginal gaps.

In the present study, OCT was able to visualize gaps of composite restorations through instant and noninvasive images, corroborating the results of other recent studies.[10,11,18-21] In previous studies, a significant increase in the signal intensity (peak) at the tooth–restoration interfacial zone was confirmed, which appeared as bright clusters and indicated the loss of marginal seal.[17]

The deterioration of the interface in this present study is in accordance with the studies mentioned previously,[10,11,18-21] which had negatively affected the tooth/restoration marginal interface compared before and after TC.

The limited depth of viewing of the OCT device, which does not allow the visualization of deeper cavities, might be a limiting factor for this type of methodology. However, the cavity size used in this study is compatible and convenient with NCCL cavities.

From the results obtained in this study, future long-term studies on the marginal integrity of NCCL and composites with respect to TC and degradation should be conducted. Future improvements related to restoration depth analyses when using OCT will allow further research. Thus, OCT can be considered a nondestructive method for evaluating the stability of the enamel/restoration and dentin/restoration interfaces simultaneously, and it has potential for clinical use.

CONCLUSION

According to the results, the following can be concluded:

- TC affected marginal integrity only for dentin margins. The enamel/restoration interface remained stable even after TC, where enamel etching was performed before placement of either adhesive system;
- Dentin margins restored with the CSE adhesive system showed better marginal adaptation than those restored with SB2 when subjected to TC. Adhesive systems performed similarly on etched enamel;
- Resin composites did not influence the marginal integrity of NCCL restorations.
- OCT could distinguish tooth tissue (enamel and dentin) from resin composite, adhesive system and marginal gaps.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of Piracicaba Dental School – UNICA. The approval code issued for this study is #104/2012.

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.

(Accepted 14 April 2015)

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Resin Bonding to a Hybrid Ceramic: Effects of Surface Treatments and Aging

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Clinical Relevance

Etching with hydrofluoric acid should be the conditioning method of choice for Vita Enamic hybrid ceramic since this surface treatment presented the highest values of bond strength after aging.

SUMMARY

The aim of this study was to verify the effects of different surface treatments on the microtensile bond strength between resin cement and a hybrid ceramic. Thirty-two hybrid ceramic slices (8 × 10 × 3 mm) were produced and allocated among four groups according to the surface treatment: Cont = no treatment, HA = 10% hydrofluoric acid applied for 60 seconds, PA = 37% phosphoric acid applied for 60 seconds and CJ = air abrasion with silica particle coated alumina (Cojet Sand, 3M ESPE, 30 μm/2.8 bar). As a control group, eight blocks of feldspathic ceramic (8 × 10 × 3 mm) were etched by hydrofluoric acid for 60 seconds (VMII). After the surface treatments, the ceramic slices were silanized (except the Cont group) and adhesively cemented to composite resin blocks (8 × 10 × 3 mm) with a load of 750 g (polymerized for 40 seconds each side). The cemented blocks were cut into beams (bonded surface area of ~1 mm^2). Half of the beams were aged (thermocycling of 5°C-55°C/6000 cy-

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DOI: 10.2341/15-057-L

cles + water storage at 37°C/60 days), and the other half were tested immediately after being cut. Data were analyzed by Kruskal-Wallis and Dunn tests (non-aged groups) and by one-way analysis of variance and Tukey test (aged groups; α=0.05%). The mode of failure was classified by stereomicroscopy. The surface treatment significantly affected the bond strength in each set of groups: non-aged (p=0.001) and aged (p=0.001). Before being aged, samples in the CJ, HA, and PA groups achieved the highest bond strength values. However, after being aged, only those in the HA group remained with the highest bond strength values. Adhesive failure was found most often. In conclusion, hydrofluoric acid etching should be used for surface conditioning of the studied hybrid ceramic.

INTRODUCTION

All-ceramic and indirect composite restorations have been widely used in recent years due to their esthetic properties and biocompatibility when compared with those of metal-ceramic restorations.[1,2] Moreover, the possibility of less invasive restorations and the enhancement of computer-aided design/computer-aided manufacturing (CAD/CAM) technology has expanded the use of such materials. An array of ceramics is commercially available, such as the feldspar-, leucite-, lithium disilicate–, alumina- and zirconia-based ceramics.[3] Several types of indirect composite materials with different filler particles are also offered.[4] Recently, a new category of ceramic/polymer material for CAD/CAM systems was developed.[5] This combination of materials apparently improves load distribution and creates a toughening mechanism that reduces crack propagation.[5]

Regarding the cementation process, the choice of a protocol is dependent on ceramic composition. The etchable ceramics—mainly the glass ceramics—have a well-defined cementation protocol that consists of hydrofluoric acid etching, silanization, and the use of a resin cement.[3,6] The etching process dissolves the glass matrix of the ceramic surface, improving micromechanical bonding.[7,8] The silane coupling agent is a bifunctional molecule that enables it to link itself to inorganic (silicon oxide) and organic (methacrylate groups of the resin cement) substances.[8,9] This cementation process enhances the mechanical behavior and the clinical performance of all-ceramic restorations by the penetration of the resin cement into the microporosities created by etching.[10,11] The indirect composite resins, in turn, can be conditioned with air-particle abrasion followed by silanization to ensure adequate union between the materials.[12]

The so-called hybrid ceramic material (Vita Enamic, Vita Zahnfabrik, Bad Säckingen, Germany), recently made commercially available, is a polymer-infiltrated ceramic network (PICN) whose composition is approximately 14% resin embedded in 86% of a ceramic network (manufacturer's information). Therefore, this material has a hybrid surface that could be conditioned in the same way as either an indirect composite or an etchable ceramic. According to a recent study,[13] the hardness of this material was provided by the ceramic content since the indenter is more susceptible to reaching this portion of the material. Therefore, it is to be expected that the ceramic content "guides" the surface treatment in the cementation process, but there are no studies reporting this.

Furthermore, it is important to consider the aging of the interfaces between hybrid ceramics and resin cement. Storage can influence the longevity of the restorations due to the small molecular size and the high molar concentration of the water, which can penetrate small spaces between polymer chains or functional groups, resulting in a decreased thermal stability of the polymer and causing its plasticization.[14]

Thus, the aim of this study was to evaluate the bond strength between a new hybrid ceramic material and a resin cement after various surface treatments with or without aging. The hypotheses were that the bond strength values would be affected by 1) the surface treatments and 2) the aging protocol used.

METHODS AND MATERIALS

The materials used in this study, with their commercial names and manufacturers, are shown in Table 1.

Specimen Preparation

Ten blocks of the ceramic materials (Vita Enamic and Vita Block Mark II, Vita Zahnfabrik) were cut into slices ($8 \times 10 \times 3$ mm^3) with a cutting machine (Isomet, Buehler, Düsseldorf, North Rhine, Germany), that were polished with SiC sandpapers (#800, #1000, #1200, Buehler) under water coolant irrigation in a polishing machine (EcoMet 300 Pro, Buehler). Silicone impressions (Elite HD, Zhermack, Badia Polesine, Rovigo, Italy) were used to create molds of these slices, and composite blocks (Filtek

Table 1: *Materials Used in This Study, With Their Commercial Names, Manufacturers, and Batch Numbers*

Type	Commercial Name	Manufacturer	Batch Number
Hybrid ceramic	Vita Enamic	Vita Zahnfabrik	36660
Feldspathic ceramic	Vita Block Mark II	Vita Zahnfabrik	35370
Hydrofluoric acid 10%	Condac	FGM	060912
Phosphoric acid 37%	Condac 37	FGM	240113
Alumina coated by silica particles of 30 μm	CojetSand	3M ESPE	411974
Silane	Clearfil Bond SE Primer and Clearfil Porcelain Bond Activator	Kuraray	01143A 00270A
Adhesive system	ED primer (A and B solutions)	Kuraray	00310A 00184A
Resin cement	Panavia F2.0	Kuraray	00255A 00033A
Composite resin	Filtek Z350 XT	3M ESPE	1314700583

Z350 XT, 3M ESPE, Seefeld, Germany) were then constructed and photoactivated for 40 seconds (Radii-Cal, SDI, Melbourne, VIC, Australia; 1200 mW/cm^2). After this first polymerization, the resin slice was removed from the mold, and the side in contact with the silicone and the other sides of the slice were polymerized for 40 seconds each.

The cementation surfaces of the blocks were conditioned according to the groups (n=8):

CONT: hybrid ceramic blocks (Vita Enamic), received no surface treatment and served as the negative control group.

PA: hybrid ceramic blocks (Vita Enamic), were etched with 37% phosphoric acid for one minute and rinsed with distilled water for one minute.

HA: hybrid ceramic blocks (Vita Enamic), were etched with 10% hydrofluoric acid for one minute and rinsed with distilled water for one minute.

CJ: hybrid ceramic blocks (Vita Enamic), were air abraded with 30-μm particles of alumina coated by silica particles for 20 seconds, with 2.8 bar of pressure and 10 mm of perpendicular distance between the air-abrasion device tip and the material surface.

VMII, feldspathic ceramic blocks (Vita Block Mark II), were etched with hydrofluoric acid at 10% hydrofluoric acid for one minute and rinsed with distilled water for one minute. This group was used as a positive control.

After all surface treatments, the ceramic blocks were ultrasonically cleaned (Cristófoli, Campo Mourão, Paraná, Brazil) in distilled water (five minutes) and air-dried for 60 seconds. The silane (Clearfil Porcelain Bond Activator and Clearfil SE Bond Primer, Kuraray Medical Inc, Okayama, Japan) was mixed and applied to the treated surface with a microbrush (except for the control group) and gently air-dried for 60 seconds. The resin cement (Panavia F, Kuraray Medical) was mixed according to the manufacturer's recommendations and applied to the ceramic surface. The composite resin blocks were cemented above the ceramic slice with a load of 750 g, the excess cement was removed with a microbrush, and the cement was polymerized for 40 seconds on each side (Radii-Cal, SDI; 1200 mW/cm^2).

Microtensile Specimen Preparation

The ceramic/composite blocks were fixed to a cylindrical metallic base coupled to a precision saw (Isomet 1000, Buehler) by means of cyanoacrylate (Super-Bonder Gel, Loctite, São Paulo, Brazil). This block was sectioned in the x- and y-axes to produce bar-shaped specimens characterized by a non-trimmed interface with a cross-sectional adhesive interface area of 1 mm^2.

Aging Protocol

After being sectioned, each ceramic/composite block resulted in 24 bar-shaped specimens. Half of these specimens were tested immediately, and the rest were subjected to an aging protocol. Samples were subjected to a combination of thermocycling and water storage. The thermocycling occurred in a thermocycling machine (Ethik Technology, São Paulo, Brazil) for 6000 cycles at 5°C/55°C. The immersion time of each bath was 30 seconds, and transfer time between the two baths was two seconds. The samples were then stored for 60 days immersed in distilled water at 37°C before being tested.

Microtensile Bond Strength Test

The bar-shaped specimens were glued to the adapted device and subjected to the microtensile bond strength (μTBS) test (Emic DL1000, Emic, São José dos Pinhais, Brazil) at a speed of 1 mm/min until fracture. The calculated μTBS (in MPa) of each

Table 2: *Bond Strength (MPa) Means and Medians and Contact Angles of the Experimental Groups*[a]

Groups	Bond Strength (MPa)				Contact Angles (Degrees)
	Non-aged		Aged		
	Mean (SD)	Median (Q1-Q3)[b]	Mean (SD)[c]	Median (Q1-Q3)	
CONT	21.96 (2.99)	22.45 (18.82-24.69) C	7.64*	—	92.00
HA	47.14 (8.10)	45.81 (39.32-55.73) AB	34.35 (2.13) a	34.54 (32.22-36.32)	82.79
PA	41.32 (13.02)	38.97 (30.48-49.75) AB	10.75 (6.39)*	9.43 (6.11-15.39)	87.31
CJ	63.25 (8.57)	63.39 (55.46-70.77) A	13.65 (4.30) c	13.22 (9.75-17.22)	5.54
VMII	34.17 (4.82)	34.99 (30.52-36.74) BC	24.18 (4.45) b	24.61 (19.57-27.14)	18.98

[a] *An asterisk indicates a mean that was not included in the statistical analysis.*
[b] *Medians that do not share the same letters are statistically different.*
[c] *Means that do not share the same letters are statistically different.*

specimen was the average between the load at failure (N) and surface area of the adhesive interface (mm^2) measured before the test.

Failure Analysis

All specimens were evaluated by stereomicroscope (Discovery Z-20, Zeiss, Jena, Germany; 75×). The failure modes were classified as adhesive (between ceramic and cement), cohesive of the cement, cohesive of the ceramic, or mixed (adhesive + cohesive). Representative specimens were observed by scanning electron microscopy (SEM; Inspect S50, FEI Company, Eindhoven, Netherlands; 190×). The specimens were cleaned with distilled water in an ultrasonic bath and air-dried. Afterward, the specimens were fixed on an aluminum stub with a carbon double-sided tape and were metalized with a gold thin conductive layer (80 Å) deposited via sputtering.

Contact Angle

For contact angle analysis, one slice of each material was used after the surface treatment proposed for each group. The contact angle was measured by a goniometer (Thetalite II, Biolin Scientific Inc, Baltimore, MD, USA) in a controlled-temperature environment. The goniometer was connected to a computer equipped with specific software (One Attension, Biolin Scientific), and the sessile drop technique was used.

A drop of distilled water was placed on the ceramic surface by means of a syringe, and after 10 seconds, the contact angle was measured for 10 seconds (30 frames per second).

Roughness Analysis

The surface roughness after the surface treatments was measured by a digital optical profilometer (Wyko, Model NT 1100, Veeco, Tucson, AZ, USA). The obtained values (Ra) corresponded to the mean of peaks and valleys. For each surface treatment, a mean value was obtained from five measurements.

Micromorphology of the Conditioned Surfaces

For analysis of the conditioned surfaces, specimens were viewed under 2000× magnification (Inspect S50, FEI Company).

Data Analysis

Data for μTBS were subjected to Kruskal-Wallis and Dunn tests (non-aged groups) and to one-way analysis of variance and Tukey test (aged groups; α=0.05%). For this, Minitab Statistical Software version 16.2.4.0 was used. The level of significance was 5%.

RESULTS

Due to the low bond strength of the CONT and PA groups, only a few specimens could be tested after aging; therefore, these groups were not included in the statistics.

Table 2 shows the μTBS values for the aged and non-aged sets of groups. For the two conditions, non-aged (p=0.001) and aged (p=0.001), the "surface treatment" was statistically significant. The median, maximum, minimum, and first and third quartiles (Q1 and Q3) of the non-aged groups are shown in the box plot (Figure 1). Among the non-aged groups, CJ attained the higher μTBS mean value. However, after the aging protocol, the HA promoted the highest adhesion.

After the aging protocol, the CONT and PA groups showed many pretest failures (Figure 2). After μTBS testing, almost all failures were adhesive between ceramic and resin cement (Figure 2). Figure 3 shows the most representative failures.

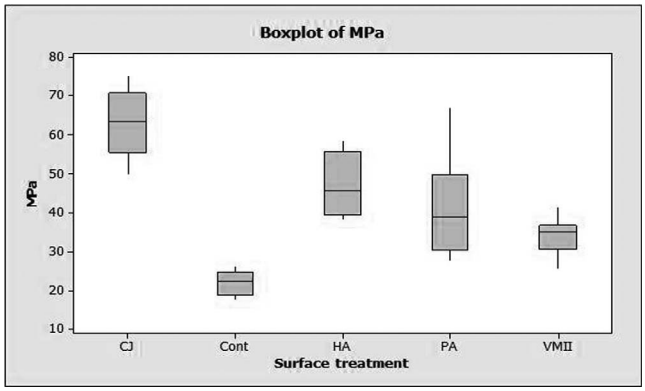

Figure 1. Box-plot graph of the non-aged groups, where the upper and lower vertical lines represent the highest and lowest retention values, respectively. The upper and lower lines of the box represent 75th and 25th percentiles, respectively. The horizontal line represents the median.

The contact angles of treated Enamic specimens were higher than those of Vita Mark, except for the CJ (Table 1). Figure 4 presents the SEM and profilometer 3D images of the conditioned surfaces.

DISCUSSION

In the present study, regarding the surface treatments, it could be seen that this factor affected the μTBS values; thus, the first hypothesis was accepted. In addition, the aging protocol significantly decreased the bond strength; thus, the second hypothesis was also accepted.

The PICN material used in this study has an interconnected structure with a dominant ceramic network containing minor composite content.[15] Consequently, the surface treatments proposed were indicated for adhesive cementation of etchable ceramics, such as hydrofluoric acid etching,[3,11] or composite indirect restorations, such as air-particle abrasion.[16,17] In this sense, as could be expected, the ceramic content of the hybrid material would "guide" the surface treatment; consequently, hydrofluoric acid etching was the most reliable treatment in this study. The amorphous ceramic material seems to be one of the so-called etchable ceramics. The glass content of this kind of ceramic suffers a selective dissolution when exposed to hydrofluoric acid, increasing the surface roughness and promoting a better micromechanical interlocking with the resin cement.

In the present study, the PICN material etched by hydrofluoric acid attained bond strength values higher than those of the similarly treated feldspathic material. This could be explained by the use of a silane with monomers in the formulation, which may have enhanced the union between the likely entirely reacted composite content of the PICN material and the monomers of the resin cement. This improvement occurs, for example, in the repair of aged composite restorations, without unreacted methacrylate groups, where a layer of an unfilled resin acts as a preparing agent whose effect is a better union between the aged and the new composites.[18] Conversely, the application of an unfilled resin layer to etched feldspathic ceramics does not appear to improve resin bonding.[11]

The air-particle abrasion with alumina particles coated by silica produced higher bond strength values among the non-aged groups. However, this treatment was severely affected by the aging protocol adopted in the current study (unstable bond). Indeed, air-particle abrasion is a surface

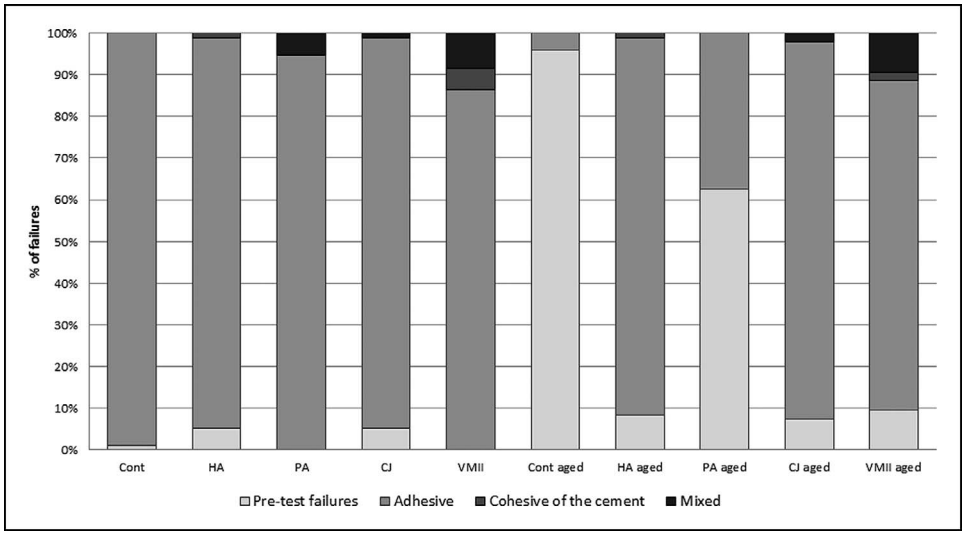

Figure 2. *Graphic representation of the failure types.*

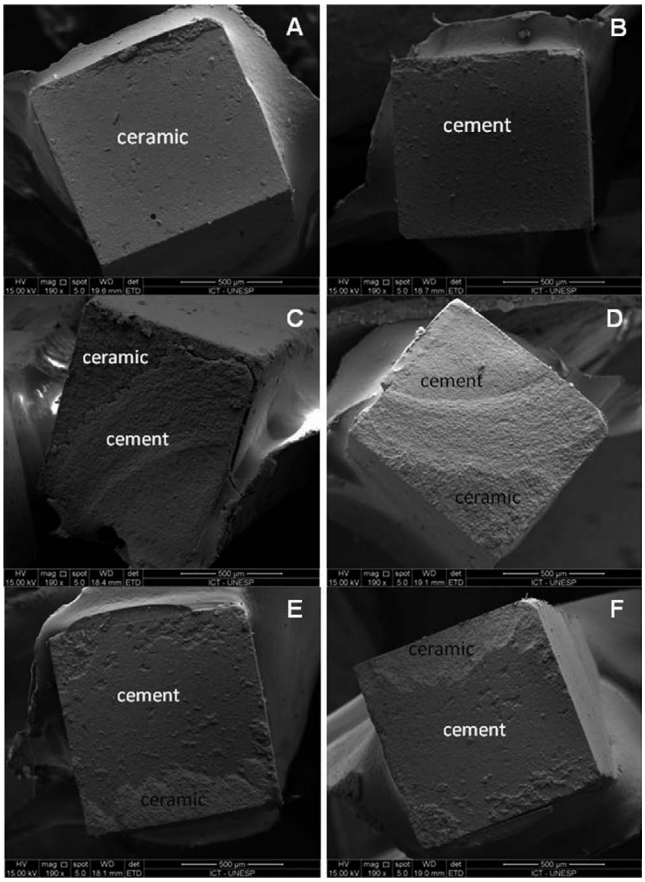

Figure 3. Micrographs of the failure types, magnification of 190×. Opposite sides of the same beam. (A,B): Adhesive failure between ceramic and cement. (C,D): Cohesive failure of the cement. (E,F): Mixed failure (adhesive + cohesive).

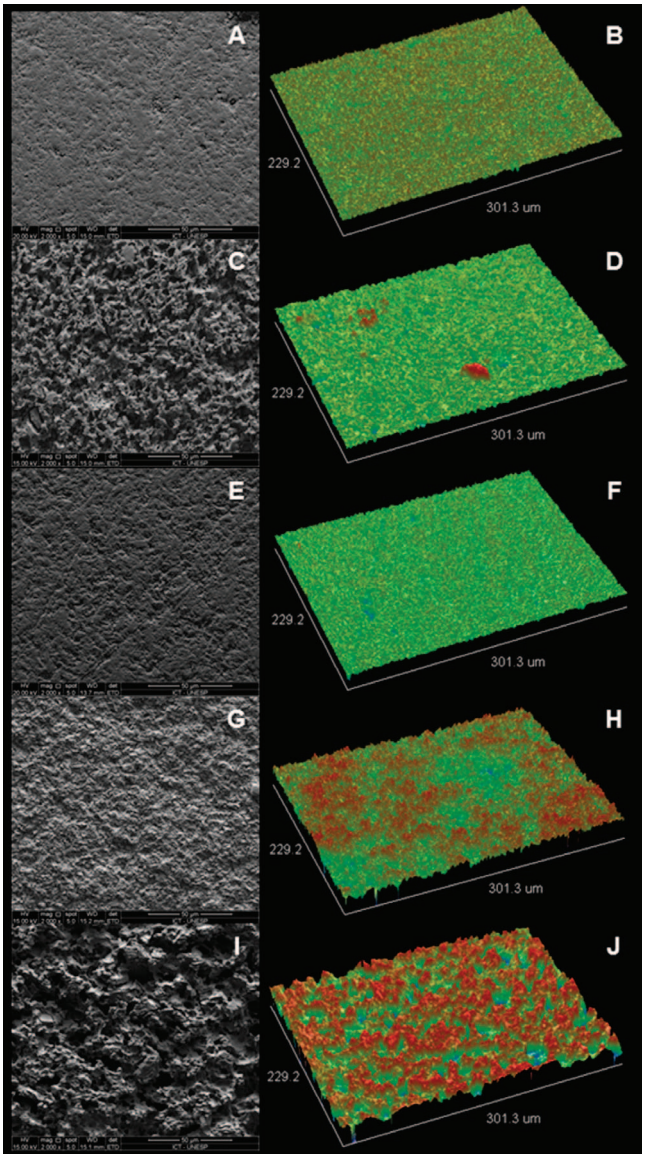

Figure 4. (A-J): Micrographs (2000×) and 3D images of the surface roughness after the surface treatments. (A,B): Cont group, Ra = 0.24 μm. (C,D): HA group, Ra = 1.32 μm. (E,F): PA group, Ra = 0.35 μm. (G,H): CJ group, Ra = 0.86 μm. (I,J): VMII group, Ra = 2.72 μm.

treatment suitable for ceramics and composites.[19] This abrasion consists of throwing some particles under pressure against the material surface, producing a more irregular surface. The increase in surface roughness caused by the air abrasion augments the interlocking between the resin cement and the ceramic.[20] In addition, when an alumina coated by silica particles is used, the impact generated by the air abrasion promotes the silicatization of the surface by a tribochemical reaction. Even though it is a surface treatment specifically indicated for nonetchable ceramics and indirect composites, it is not the best surface treatment for etchable ceramics since it could cause microcracks in the ceramic surface, which could lead to premature failures. Further, the hybrid ceramic includes glass in its composition; thus, the silicatization of the surface is not necessary for a better chemical interaction. Regarding the micromechanical interaction, Figure 4 shows that hydrofluoric acid is more powerful for increasing the roughness of the hybrid ceramic than is silicatization. Therefore, after water storage and the changes in temperature, this union between the ceramic portion of the hybrid material and resin cement may not have supported the challenge imposed. The aging protocol decreases the adhesion due to the small molecular size and high molar concentration of the water, which can penetrate small spaces between polymer chains or functional groups, resulting in a decreased thermal stability of the polymer and causing its plasticization.[14] Thus, it is possible that the polymer present in the material could not withstand the moisture and the temperature variations.

The other treatments used in this study also showed extensive deterioration of the bond strength after the aging protocol. The phosphoric acid was used only to produce a cleansing effect, providing better adhesion, even without modification of the surface topography.[21] In the SEM images, it could be seen that the surface of the PICN material after phosphoric acid application is very similar to that where the material was simply cleaned with distilled water in an ultrasonic bath. The surface roughnesses for those groups were also very similar. Thus, the resin bonding to the hybrid ceramic cannot rely solely on surface decontamination, and, as previously stated by other authors,[10,11] the ceramic and the composite surface should not adhere to the resin cement unless surface alterations are made on the material (increase in roughness) with consequent mechanical bonding.

The aging protocol used in this study was capable of decreasing the bond strength values of all the surface treatments proposed. Considering the types of failure found after analysis by stereomicroscopy, it is possible to affirm that fracture occurred mostly in the adhesive zone, while cohesive failures were less frequent, which benefits the real evaluation and interpretation of bond strength data.[22]

When the surface treatments of the hybrid ceramic HA and VMII were compared, the latter presented a lower contact angle and higher roughness but lower values of bond strength, demonstrating that the bond mechanism of the VMII appears to depend more on micromechanical interlocking, while the hybrid ceramic appears to depend more on the chemical bond for the reasons explained previously. In comparisons of only the surface treatments of the hybrid ceramics, the CJ and HA groups showed the lowest contact angle and the highest roughness, respectively. After being aged, samples in the HA group showed the highest values of bond strength. In fact, the contact angle values are influenced by the surface topography, the surface tension of the liquid, and the surface energy of the solid substrate. This value can vary according to the level of interaction between the liquid and the solid.[23,24] Thus, even though the contact angle was high when distilled water was used, the silane coupling agent may have changed the materials' interactions, improving bond strength.

Furthermore, SEM micrographs revealed prominent undercuts and honeycomb-shaped surface irregularities in HA specimens (Figure 1C,D). In contrast, the sandblasting of the CJ group under high pressure generated sharply demarcated, acute-angled surface features caused by spallation of small areas of the glass matrix. This was confirmed through the SEM image of the HA group, which appeared much rougher than the sandblasted ceramic surfaces (Figure 1G,H).

One of the limitations of this study was the pretest failures, which were dominant in the CONT group after aging. However, it was evident that although the hybrid ceramic includes resin in its composition, it requires surface treatment for bonding longevity at the interface. Another limitation was that only one brand of hybrid ceramic was included in this study; subsequently, the results presented here are validated for Vita Enamic material only and should not be extrapolated to other brands of hybrid ceramic.

The relevance of this study was that it simulated different surface treatments for the new hybrid ceramic. Further studies should be conducted to investigate other factors involved in the retention of crowns with this material, such as longitudinal fatigue testing and the evaluation of different cementation strategies.

CONCLUSIONS

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

- Surface treatment with hydrofluoric acid improves the bond strength between the hybrid ceramic studied and resin cement.
- Aging was associated with lower bond strengths for all surface treatments.

Regulatory Statement

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

Conflict of Interest

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

(Accepted 16 May 2015)

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Strengthening of Porcelain Provided by Resin Cements and Flowable Composites

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FB Leal • L Correr-Sobrinho • RR Moraes

Clinical Relevance

Resin coating and use of resin-based luting agents with better physical properties generally improve the mechanical performance of porcelain.

SUMMARY

This study evaluated the effect of mechanical properties of resin-based luting agents on the strength of resin-coated porcelain. The luting agents tested were two flowable resin composites (Filtek Z350 Flow and Tetric-N Flow), a light-cured resin cement (Variolink Veneer [VV]), and a dual-cured resin cement (Variolink II) in either light-cured (base paste) or dual-cured (base + catalyst pastes [VD]) mode. Flexural strength (σ_f) and modulus of elasticity (E_f) of the luting agents were measured in three-point bending mode (n=5). Porcelain discs (Vita VM7) were tested either untreated (control) or acid etched, silanized, and coated with the luting agents. Biaxial flexural strength (σ_{bf}) of the porcelain discs was tested using a ball-on-ring setup (n=30). The σ_{bf} of the resin-coated specimens was calculated at z-axial positions for multilayer specimens in the ball-on-ring test: position $z = 0$ (ceramic surface at the bonded interface) and position $z = -t_2$ (luting agent surface above ring). The σ_f and E_f data were subjected to analysis of variance and the Student-Newman-Keuls test (α=0.05). A Weibull analysis was performed for σ_{bf} data. Weibull modulus (m) and characteristic strength (σ_0) were calculated. Linear regression analyses investigated the relationship between mechanical properties of the luting agents and the strengthening of porcelain. VD had higher and VV had lower mechanical strength than the other materials. At $z = 0$, all resin-coated groups

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DOI: 10.2341/15-025-L

had higher σ_{bf} than the control group. No significant differences between the luting agents were observed for σ_{bf} and σ_0. At $z = -t_2$, VD had the highest σ_{bf} and σ_0, whereas VV had the poorest results. No significant differences in m were observed across groups. A linear increase in flexural strength of the porcelain was associated with increased σ_f and E_f of the luting agents at position $z = -t_2$. In conclusion, resin coating and use of luting agents with better physical properties generally improved the mechanical performance of porcelain.

INTRODUCTION

Feldspathic porcelains are intrinsically fragile but might obtain additional strengthening when cemented to the dental structure using resin-based luting agents.[1,2] Feldspathic porcelain veneers thinner than 1 mm can be adhesively bonded to tooth tissues and present high clinical survival, with the adhesive cementation playing an important role on the clinical performance of these restorations.[3]

The strengthening of ceramics provided by bonding has been linked to mechanisms that include crack healing by resin infiltration[4] and induction of crack closure stresses by the polymerization shrinkage of resin-based agents.[5] However, *in vitro* investigations have demonstrated that the traditionally accepted hypotheses do not adequately describe the strengthening patterns observed.[1] Novel strengthening mechanisms insensitive to individual defect severity but sensitive to the surface texture have been identified whereby the strengthening is dependent on the creation of an interpenetrated resin-ceramic hybrid layer.[1,6] The magnitude of the strengthening has been also suggested to be dependent on the modulus of elasticity (E_f) of the resin-based luting agent.[6]

Commercially available resin-based luting agents present considerable variability regarding their interaction mechanism with dental tissues, formulation of organic and inorganic phases, and curing modes. All these factors might influence the characteristics of the polymer formed and confer different mechanical properties to the luting material as well as for the bonded veneer.[7] Light-cured resin-based luting agents are usually indicated for bonding porcelain veneers due to their improved color stability compared to dual-cured agents and for the high degree of C=C conversion achieved on light activation. Flowable resin composites and dual-cured resin cements are also available for the same purpose.[8] However, the performance of the resin-based luting agents with distinct polymerization modes on strengthening of porcelain veneers has not received attention. Different resin luting agents intrinsically have distinct physical properties that might impact the strengthening of porcelain on cementation.

The aim of this study was to evaluate the effect of flexural strength (σ_f) and E_f of different commercially available resin-based luting agents on the strengthening of porcelain. The hypothesis tested was that resin-based luting agents with higher E_f and σ_f would provide higher strengthening for the porcelain.

METHODS AND MATERIALS

Mechanical Properties of the Resin-Based Luting Agents

The resin-based luting agents tested in the study are presented in the Table 1. The E_f and σ_f of the luting agents were measured in three-point bending mode. Bar-shaped specimens ($25 \times 2 \times 2$ mm) of each luting agent (n=5) were obtained using a split metallic mold covered with Mylar strip. Light curing was carried out according to the International Organization for Standardization Standard 4049 [9] using a light-emitting diode curing unit (Bluephase, Ivoclar Vivadent, Schaan, Liechtenstein) with irradiance of 1100 mW/cm^2, which was monitored throughout the experiment. For the VL group, only the base paste of Variolink II was used, whereas for the VD group, both base and catalyst pastes of Variolink II were mixed before use. The specimens were stored in distilled water at 37°C for 24 hours. The flexural test was performed on a mechanical testing machine (model 4411, Instron, Canton, MA, USA) at a crosshead speed of 1 mm/min. The σ_f and E_f were calculated by computer software (Blue Hill 2, Instron) according to the following equations:

$$\sigma_f = \frac{3PL}{2bd^2} \quad (1)$$

$$E_f = \frac{PL^3}{4bd^3D} \quad (2)$$

where P is the load (N), L the support span (20 mm), b the width (mm), d the specimen thickness (mm), and D the deflection (mm).

Preparation of Porcelain Discs

A total of 180 disc-shaped porcelain specimens were obtained. The porcelain powder (VM7 Transpa Dentine 2M2, batch no. 30270, Vita Zahnfabrik,

Table 1: *Description of the Resin-Based Luting Agents Tested*

Material (Group Code)	Manufacturer	Composition[a]	Filler Content	Batch
Filtek Z350 Flow (ZF)	3M ESPE (St Paul, MN, USA)	Bis-GMA, TEGDMA, Bis-EMA dimethacrylate polymer, silane-treated ceramic, silane-treated silica, ytterbium trifluoride, titanium oxide	65 wt% 55 vol%	N144977
Tetric-N Flow (TF)	Ivoclar Vivadent (Schaan, Liechtenstein)	Dimethacrylates (including TEGDMA), inorganic fillers, catalysts, stabilizers, pigments	63 wt% 39 vol%	M63678
Variolink Veneer (VV)		Dimethacrylates, inorganic filler, catalysts, stabilizers, pigments	60.1 wt%[b] 40 vol%	M37825
Variolink II, light cured (VL)		BisGMA, UDMA, TEGDMA	Base paste: 73.4 wt% 46.7 vol%	N53689
Variolink II, dual cured (VD)		Bis-GMA, UDMA, TEGDMA, inorganic fillers, catalysts, stabilizers, and pigments	Base paste: 73.4 wt% 46.7 vol%	N01558
			Catalyst paste: 77.2 wt% 52 vol%	N16895

Abbreviations: Bis-GMA, bisphenol-A glycidyl dimethacrylate; TEGDMA, triethyleneglycol dimethacrylate; UDMA, urethane dimethacrylate.
[a] *As disclosed by the manufacturers.*
[b] *As quoted by Ozturk and others.*[20]

Bad Säckingen, Germany) was mixed with the modeling liquid (VM Modelling Liquid, batch no. 34240, Vita) to produce a thick slurry and condensed into a metallic mold (15-mm diameter, 0.9-mm thickness). The mold was overfilled and placed on a vibrating table for 90 seconds, and excess liquid was removed with an absorbent tissue. The surface was leveled with a razor blade to produce discs of uniform thickness. Each disc was removed carefully from the mold, placed on a refractory substrate, and fired in a ceramic furnace (Vacumat 40, Vita) according to the manufacturer's directions. The discs were cooled to room temperature and visually inspected. Discs with defects or visible cracks were discarded and replaced. The discs were manually wet ground on both sides with 320-grit SiC abrasive papers (Norton S.A., São Paulo, Brazil) to produce flat surfaces 0.8 ± 0.1 mm in thickness. Wet polishing was performed with 600- and 1200-grit SiC abrasive papers for 60 seconds on each surface. Final dimensions were checked with a digital caliper (Absolute Digimatic, Mitutoyo, Tokyo, Japan), and the porcelain discs were randomly divided into six groups (n=30) according to the resin-based luting agent tested. Untreated porcelain discs (no luting agent) were tested in the control group.

Resin Coating of Porcelain Discs

The ground surfaces of the porcelain discs were etched with 10% hydrofluoric acid for 90 seconds (batch no. 479058D, Dentsply Caulk, Milford, DE, USA), washed for 60 seconds, and dried with water- and oil-free compressed air for 30 seconds. Two thin silane layers (RelyX Ceramic Primer, batch no. N136724, 3M ESPE, St. Paul, MN, USA) were applied and dried after 60 seconds with compressed air for 30 seconds. The resin-based luting agents were manipulated following the manufacturer's instructions, and a standard mass was applied onto the center of each disc. For the VL group, again only the base paste was used, whereas for the VD group, both base and catalyst pastes were used. A glass coverslip was lightly pressed to extrude the luting agent, and the resulting coverslip/luting agent/porcelain assembly was transferred to a leveled loading platform. The resin–porcelain specimens were centrally oriented, a controlled load of 5 N was applied, and excess luting agent was removed. Light curing was carried out for 60 seconds through the porcelain with the light guide tip positioned at the center of the disc. The specimens from the VD group also were light cured. The thickness of the luting agent layer applied to porcelain was measured, and resin–porcelain specimens with luting agent thickness outside the range between 100 and 150 μm were discarded and replaced by new resin–porcelain specimens. The specimens were dry stored for 24 hours in lightproof containers at 37°C.

Biaxial Flexural Strength (σ_{bf}) Test

The σ_{bf} of the porcelain discs (control group) and resin–porcelain specimens was determined on the mechanical testing machine using a ball-on-ring setup. The discs were centrally placed on a 10-mm-diameter knife-edged support and loaded with a spherical indenter (4-mm diameter) at a crosshead speed of 1 mm/min. A thin section of rubber dam sheet was placed between the support and specimen to accommodate slight distortions in specimen geometry.[1,2,4,6,14] The σ_{bf} (MPa) for the porcelain discs (control group) was calculated using the following equation[10,11]:

$$\sigma_{bf} = \frac{3P(1+v)}{4\pi t^2}\left[1 + 2ln\left(\frac{a}{b}\right) + \frac{1-v}{1+v}\left[1 - \frac{b^2}{2a^2}\right]\frac{a}{R^2}\right] \quad (3)$$

where P is the fracture load (N), v the Poisson ratio (0.25) of the porcelain,[12] t the disc thickness (mm), a the radius of the knife-edged support (mm), b the radius of uniform loading at center (mm), and R the radius of the disc-shaped specimen (mm).

The σ_{bf} of the resin–porcelain specimens was calculated according to the analytical solutions described by Hsueh and others[13] and used in previous studies.[1,2,6,14,15] First, the modulus of elasticity of the porcelain (E_1^*) and resin-based luting agent (E_2^*) were calculated as a function of the Poisson ratio of the porcelain and luting agent:

$$E_1^* = \frac{E_1}{1-v_1^2} \qquad E_2^* = \frac{E_2}{1-v_2^2} \quad (4)$$

where E_1 is the modulus of elasticity of the porcelain,[12] E_2 is the measured modulus of elasticity of the resin-based luting agents, and v_1 and v_2 are the Poisson ratios of the porcelain (0.25)[12] and luting agents (0.27), respectively.[16] Thereafter, the neutral plane (tn) of the resin–porcelain specimens was calculated as a function of the porcelain and luting agent thicknesses (t_1 and t_2) and calculated moduli of elasticity (E_1^* and E_2^*), respectively:

$$tn = \frac{E_1^*(t_1)^2 - E_2^*(t_2)^2}{2(E_1^* t_1 + E_2^* t_2)} \quad (5)$$

The σ_{bf} of the resin–porcelain specimens was calculated at z-axial positions at the center of the discs, where the ceramic surface at the bonded interface was located at position $z = 0$ (equation 6) and the resin luting agent surface above the ring of the ball-on-ring setup was located at position $z = -t_2$ (equation 7):

$$\sigma_{bf} = \frac{-3P(1+v)(z-tn)}{2\pi(t_1+t_2)^3}\left[1 + 2ln\left(\frac{a}{b}\right) + \frac{1-v}{1+v}\left(1 - \frac{b^2}{2a^2}\right)\frac{a^2}{R^2}\right] \times \left[\frac{E_1^*(E_1^* t_1 + E_2^* t_2)(t_1+t_2)^3}{(E_1^* t_1^2)^2 + (E_2^* t_2^2)^2 + 2E_1^* E_2^* t_1 t_2(2t_1^2 + 2t_2^2 + 3t_1 t_2)}\right] \quad (z = 0) \quad (6)$$

$$\sigma_{bf} = \frac{-3P(1+v)(z-tn)}{2\pi(t_1+t_2)^3}\left[1 + 2ln\left(\frac{a}{b}\right) + \frac{1-v}{1+v}\left(1 - \frac{b^2}{2a^2}\right)\frac{a^2}{R^2}\right] \times \left[\frac{E_2^*(E_1^* t_1 + E_2^* t_2)(t_1+t_2)^3}{\left[(E_1^* t_1^2)^2 + (E_2^* t_2^2)^2 + 2E_1^* E_2^* t_1 t_2(2t_1^2 + 2t_2^2 + 3t_1 t_2)\right]}\right] \quad (z = -t_2) \quad (7)$$

$$v = \frac{v_1 t_1 + v_2 t_2}{t_1 + t_2} \quad (8)$$

Statistical Analysis

Mechanical data from tests with the resin-based luting agents and porcelain discs passed normality and equal variance tests. Data for σ_f and E_f of the resin-based luting agents were separately analyzed using one-way analysis of variance followed by the Student-Newman-Keuls *post hoc* test (α=0.05). A Weibull analysis was performed for the σ_{bf} data using the software Weibull++ (Reliasoft, Tucson, AZ, USA). Weibull modulus (m) and characteristic strength (σ_0) were calculated based on the maximum likelihood method, and the 95% upper and lower confidence bounds were calculated using the likelihood ratio.[2,17] The σ_{bf} and σ_0 means of the resin–porcelain specimens were plotted against the σ_f and E_f means of the resin-based luting agents; regression analyses were performed to investigate the relationship between the mechanical properties of the resin-based luting agents and the strengthening at axial positions $z = 0$ and $z = -t_2$ of the porcelain specimens.

RESULTS

Results for the mechanical properties of the resin-based luting agents are presented in Table 2. The VD group in general had significantly higher σ_f and E_f than the other materials, followed by ZF. The other luting agents had intermediate results, whereas VV generally presented significantly lower σ_f and E_f than all the other luting agents.

Table 3 presents the results for σ_{bf}, σ_0, and m for the different axial positions of the porcelain specimens ($z = 0$ and $z = -t_2$). At position $z = 0$, all resin-coated groups had significantly higher σ_{bf} than the

Table 2: *Means (Standard Deviations) for Flexural Strength (σ_f) and Flexural Modulus (E_f) of the Resin-Based Luting Agents (n=5)*

Luting Agent	σ_f, MPa	E_f, GPa
Filtek Z350 Flow	99 (10) AB[a]	4.7 (0.2) B
Tetric-N Flow	81 (12) BC	2.8 (0.4) C
Variolink Veneer	66 (10) C	2.0 (0.2) D
Variolink II, light cured	88 (13) B	3.3 (0.4) C
Variolink II, dual cured	112 (17) A	6.5 (0.5) A

[a] *Distinct letters in each column indicate significant differences between groups ($p<0.05$).*

control group. The Weibull plot for position $z = 0$ is shown in Figure 1. No significant differences between the luting agents were observed for σ_{bf} and σ_0. The Weibull modulus for all groups was similar, even for the control group. At position $z = -t_2$, the group VD had the highest σ_{bf} and σ_0, whereas the group VV the lowest σ_{bf} and σ_0. The other resin-based luting agents had intermediate results. The Weibull plot for position $z = -t_2$ is shown in Figure 2. No significant differences in m were observed across groups.

Plots of the linear regression analyses between σ_{bf} and σ_0 of the resin–porcelain specimens and flexural properties of the resin-based luting agents (σ_f and E_f) are displayed in Figure 3 (z=0) and Figure 4 (z=$-t_2$). No significant association was observed between σ_{bf} or σ_0 of the resin–porcelain specimens and properties of the resin-based luting agents at position $z = 0$. In contrast, all models showed a significant linear increase in σ_{bf} and σ_0 of the resin–porcelain specimens associated with increased σ_f and E_f of the resin-based luting agents at position $z = -t_2$.

DISCUSSION

Resin coating of the porcelain discs yielded an increase in flexural properties of around 100% in all groups regardless of the resin-based luting agent tested. The strengthening effect of resin-based luting agents on porcelain is in accordance with previous studies, suggesting that this effect occurs due to the formation of a porcelain–resin hybrid layer resulting from the interpenetration of the resin in the etched porcelain surface.[1,2,6] The mechanical properties of the resin-based luting agents can interfere with the reinforcement of porcelain. At axial position $z = 0$ (porcelain surface at bonded interface), no significant differences were observed among the different luting agents. The linear regression analysis at position $z = 0$ (Fig. 3) corroborates this finding. However, at axial position $z = -t_2$, both the biaxial flexural test and the linear regression analysis (Figure 4) indicate that resin-based luting agents with improved mechanical properties led to significantly higher flexural strength of the resin–porcelain assembly.

E_f and σ_f were in general significantly different among the resin-based luting agents evaluated. VD showed the highest results, while VV had the poorest mechanical performance. Explanation for these findings lies in the composition of the inorganic filler content of the luting agents. The resin cement Variolink II has the highest filler loading among the cements tested. VV, in contrast, has the lowest filler loading of the luting agents tested. Previous studies also have indicated a positive relationship between mechanical properties and filler content of resin-based particulate composites.[18-22] The mechan-

Table 3: *Means (95% Confidence Intervals) for Biaxial Flexural Strength (σ_{bf}), Characteristic Strength (σ_0), and Weibull Modulus (m) at Axial Positions z = 0 or z = $-t_2$ (n=30)*

	Axial Position/Group	σ_{bf}, MPa	σ_0, MPa	m
$z = 0$	Control[a]	63 (59–67) B[b]	68 (63–73) B	6.7 (4.6–9.2) A
	Filtek Z350 Flow (ZF)	134 (126–142) A	145 (135–155) A	7.2 (4.9–9.9) A
	Tetric-N Flow (TF)	126 (118–134) A	136 (125–147) A	6.0 (4.2–8.2) A
	Variolink Veneer (VV)	131 (122–140) A	142 (139–146) A	5.4 (4.5–5.4) A
	Variolink II, light cured (VL)	129 (117–141) A	142 (125–161) A	4.0 (2.8–5.4) A
	Variolink II, dual cured (VD)	133 (121–145) A	146 (129–165) A	4.1 (2.8–5.5) A
$z = -t_2$	ZF	13.0 (11.8–14.2) B	14.3 (12.6–16.2) B	4.0 (2.8–5.4) A
	TF	7.5 (7.0–8.0) C	8.1 (7.4–8.9) C	5.4 (3.7–7.5) A
	VV	5.0 (4.6–5.4) D	5.4 (4.9–5.9) D	5.2 (3.6–7.1) A
	VL	8.4 (7.5–9.3) C	9.4 (8.1–10.7) C	3.6 (2.5–4.9) A
	VD	18.6 (16.5–20.7) A	20.7 (17.9–23.8) A	3.5 (2.4–4.8) A

[a] *The control specimens were porcelain discs without luting agent, with no calculation for different z-axial positions.*
[b] *Distinct letters in each column indicate significant differences between groups (data for z=0 and z=$-t_2$ are not interrelated).*

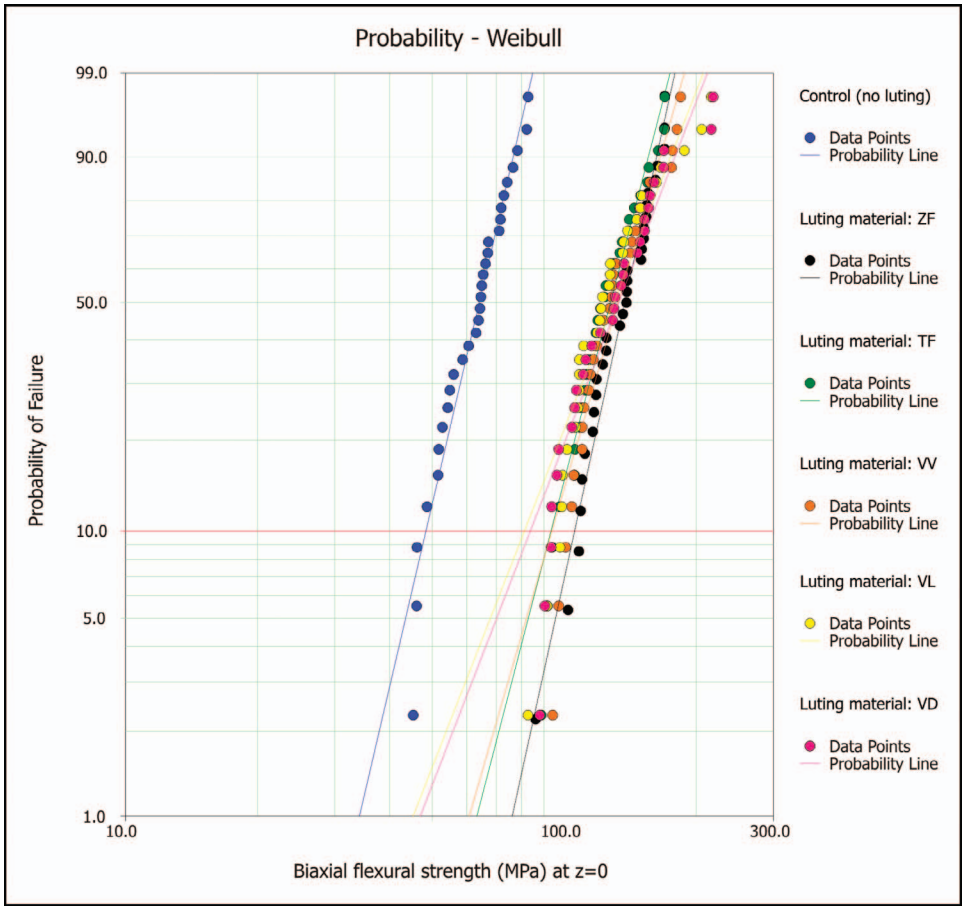

Figure 1. *Probability of failure (Weibull analysis) for all groups at axial position* z = *0.*

ical performance of composites can be improved by increasing their filler content, assuming that integrity is maintained between the resin matrix and filler particles mediated through silane coupling agent.[23] On the other hand, the viscosity will increase as well, thus limiting the film thickness of the luting material.[24]

Differences in degree of C=C conversion between the resin-based luting agents might also have a role in their mechanical strength. The dual-cured resin cement tested, for instance, had significantly higher flexural properties in the dual-cured mode (base + catalyst pastes) compared to the light-cured mode (base paste only). This finding is explained by the higher filler loading of the catalyst paste compared to the base paste, but certainly the additional redox curing contributed to the improved strength by increasing C=C conversion and polymer cross-linking. It has been shown that dual-polymerized resin cements are dependent on light curing for improved polymerization[25,26] and that the C=C conversion tends to be higher in the dual-cure than in the self-cure mode alone.[25-27] However, the present findings further suggest that the monomer conversion in the dual-cure mode might be higher than in the light-cure mode alone. Distinct degrees of C=C conversion between the light-activated luting agents tested might additionally exist because differences in the composition of the resin phase also affect the polymerization extent of resin-based luting agents.[28]

Increased strength associated with resin coating of porcelain has been identified to be linearly dependent on the E_f of resin-based luting agents.[6] It has been suggested that adhesive cementation of porcelain laminate veneers with resin-based luting agents with better mechanical performance could result in increased clinical performance.[1,6] Although this result is yet to be verified in clinical trials, findings of the present study show that at the porcelain surface in the bonded interface (position z=0), the mechanical properties of the resin-based luting agents do not have a role on the strengthening effect. In other words, the resin coating itself was preponderant over the mechanical properties of the luting agents tested at position $z = 0$. One could expect that materials with higher filler loading

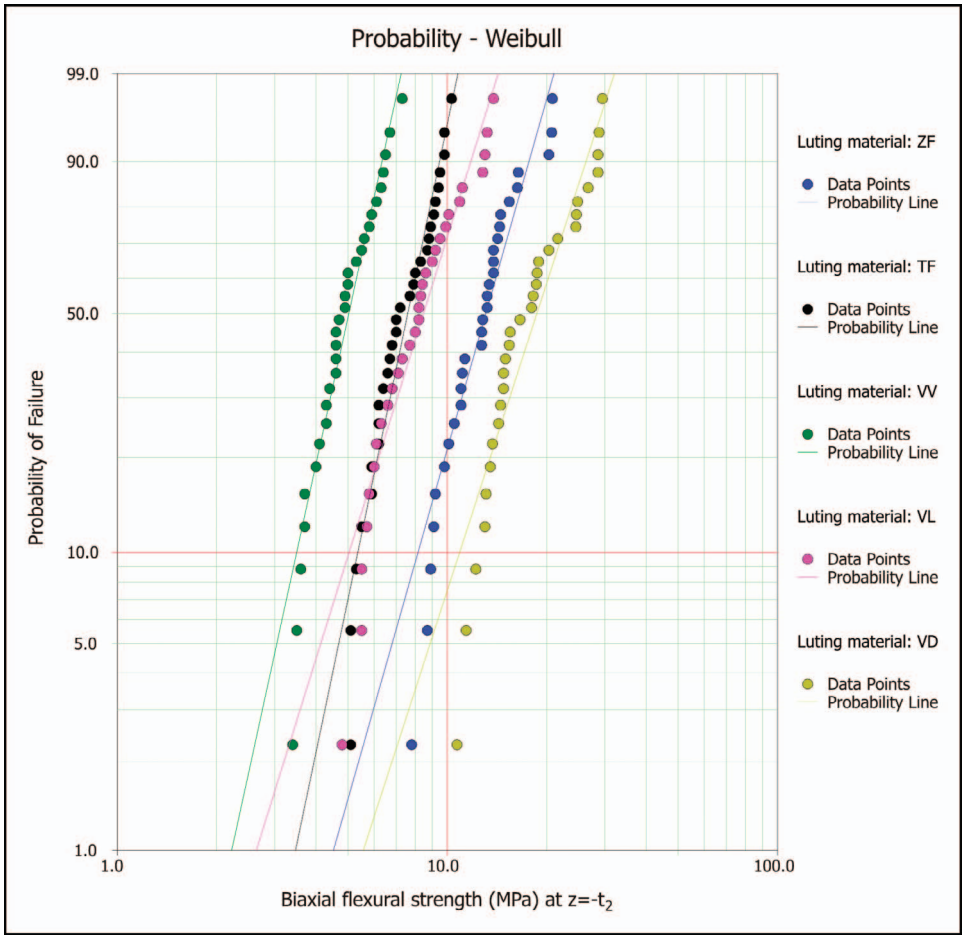

Figure 2. *Probability of failure (Weibull analysis) for all resin-coated groups at axial position* $z = -t_2$.

would positively impact the strengthening mechanism. However, highly filled composites are more viscous and may differ from less viscous materials in terms of their potential to generate intimacy between the porcelain surface defects and the infiltrating polymer.[29] In addition, the difference in E_f among the resin-based luting agents tested, although significant statistically, was not appreciable, with means varying from 2.0 to 6.5 GPa, whereas the previously quoted study evaluated luting agents with E_f varying between 4.9 and 16.8 GPa.[6] The lower range of moduli of elasticity and luting agent viscosity could explain the similar strength results at position $z = 0$. Studies using model resin-based luting agents with controlled properties could better address that issue.

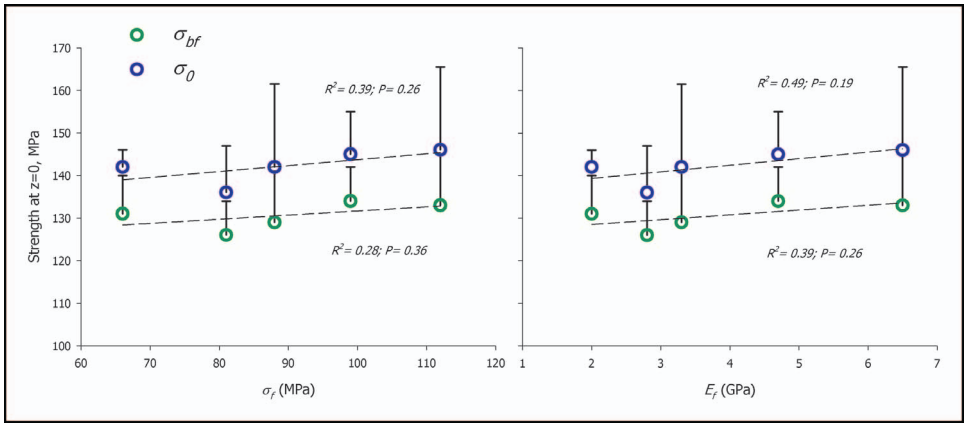

Figure 3. *Linear regression plots with stress* (σ_{bf} *and* σ_0) *at axial position* $z = 0$ *as dependent variable. Symbols are means ± 95% confidence intervals. Coefficients of linear regression* (R^2) *and their respective p-values are displayed for each condition. No significant association was observed between flexural strength of the resin–porcelain specimens and flexural properties* (σ_f and E_f) *of the resin-based luting agents.*

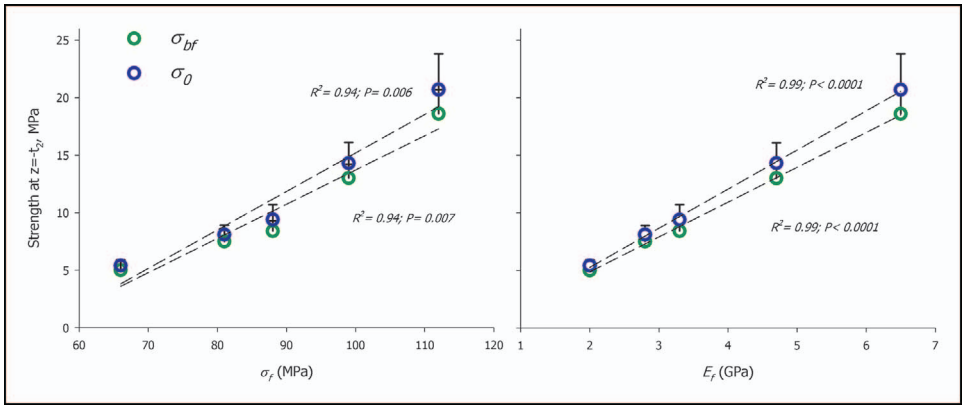

Figure 4. *Linear regression plots with stress (σ_{bf} and σ_0) at axial position z = $-t_2$ as dependent variable. Symbols are means ± 95% confidence intervals. Coefficients of linear regression (R^2) and their respective p-values are displayed for each condition. All models showed a significant linear increase in flexural strength of the resin–porcelain specimens associated with increased σ_f and E_f of the resin-based luting agents.*

In contrast to axial position $z = 0$, the mechanical properties of the resin-based luting agents were significantly associated with the strengthening at position $z = -t_2$. This finding indicates that the mechanical strength of the resin-based luting agent is indeed important for the overall mechanical performance of the bonded porcelain. This is in accordance with previous studies[1,30] that indicated that the strengthening of porcelains by resin coating was independent of a controlled defect population, although the strengthening observed was attributed to the elastic behavior of the resin. The mathematical methods used here[13] enable calculating biaxial flexure stress at axial positions throughout the resin–porcelain specimen during failure. The σ_{bf} observed at position $z = -t_2$ was considerably below the σ_f and elastic limits of the resin-based luting agents tested. This means that it is unlikely that failure during loading initiated at the resin surface or within the bulk of the luting agent, as previously proposed.[1] However, the overall porcelain strengthening provided by resin coating may be dependent on the mechanical behavior of the luting agent interpenetrating the porcelain. Luting agents with better physical properties (higher σ_{bf} at position $z=-t_2$) could better withstand the intraoral loading and maintain an adequate bonding to the tooth structure, ultimately preventing early failure of the porcelain restoration.

The resin–porcelain specimens were dry stored before being subjected to biaxial flexural testing. A previous study[2] showed that storage in water for 24 hours of specimens similar to those tested here may be detrimental to the mechanical performance of resin-coated porcelain. The study showed reductions of approximately 2% and 10% of σ_f at axial positions $z = -t_2$ and $z = 0$, respectively, and a 7.4% reduction in σ_0 at $z = 0$ for silane-treated porcelain coated with resin cement. Interesting was the fact that m of the resin cement–porcelain specimens was reduced up to 45% after short-term water storage, a finding that was attributed to hydrolytic effects acting over ceramic bonds and polymer matrix. However, the comparisons between luting materials in that study were generally not affected within dry- and wet-stored groups. In addition, the porcelain surfaces were alumina abraded before luting,[2] whereas the specimens tested here were acid etched. It is known that alumina sandblasting may lead to the creation of a surface topography more challenging for a proper infiltration of resin cements as compared to acid etching,[31,32] particularly if no unfilled resin (adhesive) is applied to the ceramic. Therefore, lower hydrolytic effects taking place in the first 24 hours after bonding to acid-etched porcelain could occur, although this effect still has to be determined.

The hypothesis tested was partially accepted because improved mechanical properties of the resin-based luting agents were associated with improved flexural strength of the luted porcelain at position $z = -t_2$ but not at position $z = 0$. As a consequence, the present results suggest that the strength and performance of resin-luted thin porcelain restorations could be enhanced by the use of resin-based luting agents with better mechanical properties. However, proper interpenetration of the resin–cement on the etched porcelain surface also is relevant. One of the limitations of the methods used here is that tooth abutments are not employed in the biaxial testing, and the interaction of the luting agent with dentin/enamel is also important for the mechanical performance of bonded porcelain. Ongoing studies using experimental resin-based luting agents with controlled mechanical properties may better address the effect of resin coating on the mechanical performance of porcelain; the results will be shown in a separate report.

CONCLUSIONS

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

- Resin coating was associated with porcelain strengthening.
- Resin-based luting agents with better mechanical properties might positively interfere with the strengthening of the resin–porcelain assembly.
- Similar mechanical performance was observed at the porcelain surface in the bonded interface for resin–porcelain specimens with different resin-based luting agents.

Acknowledgements

The study was partially supported by FAPERGS/Brazil (protocol 0902063) and CNPq/Brazil (protocol 503897/2012-4).

Regulatory Statement

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

Conflict of Interest

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

(Accepted 30 April 2015)

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Effects of Immediate Dentin Sealing and Pulpal Pressure on Resin Cement Bond Strength and Nanoleakage

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C Ely • AF Reis

Clinical Relevance

The immediate dentin sealing technique was able to prevent negative effects of pulpal pressure on interfaces produced by self-adhesive and conventional multistep resin cements.

SUMMARY

Objective: The object of this study was to evaluate the simulated pulpal pressure (SPP) and immediate dentin sealing technique (IDS) effects on the microtensile bond strength (μTBS) and nanoleakage of interfaces produced by different luting agents.

Methods and Materials: Two self-adhesive luting agents (RelyX Unicem [UC] and Clearfil SA Luting [SA]) and two conventional luting agents (Rely X ARC [RX] and Panavia F [PF]) were evaluated. Eighty human molars were divided in four groups according to luting agents. Each group was subdivided according to SPP (with or without) and dentin sealing (immediate or delayed) using Clearfil SE Bond (n=5). After IDS was performed, specimens were stored in water for seven days before luting procedures. Composite blocks were luted according to the manufacturers' instructions. One half of the specimens were subjected to 15 cm H_2O of hydrostatic pressure for 24 hours before cementation procedures and continued for 24 hours afterward. Then, restored teeth were sectioned into beams and tested in tension. Two additional teeth per group were prepared for nanoleakage evaluation with scanning electron microscopy. Bond strength data were statistically analyzed by three-way analysis of variance and Tukey test.

Results: μTBS of RX decreased when it was subjected to SPP without IDS. However, in the same conditions, μTBS of UC increased. The IDS prevented negative influence of SPP on μTBS of RX and PF; however, a decrease in

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DOI: 10.2341/15-150-L

μTBS of SA and UC was observed. Except for RX, IDS increased μTBS for all resin cements.

Conclusion: Independent of SPP, the IDS technique obtained higher μTBS for PF, SA, and UC and did not influence RX μTBS.

INTRODUCTION

The multistep adhesive cementation technique is considered complex and sensitive.[1,2] Problems that can occur during application can account for higher incidence of postoperative sensitivity after bonding procedures,[3] pulpal damage,[4] and premature degradation of the resin-dentin interface.[5] Self-adhesive luting agents do not require any pretreatment of the tooth surface and were developed in an attempt to simplify bonding procedures and reduce the shortcomings of the conventional multistep adhesive cementation technique.[6,7] Their application on smear layer-covered substrates maintains dentin permeability in very low levels,[8] contributing to reduced postoperative sensitivity and lower susceptibility to moisture degradation.[9] The interaction with subjacent dentin and enamel has been suggested to occur through formation of a hybridized complex and chemical interaction with hydroxyapatite.[10]

Dentin is the main substrate available for adhesion in prosthetic procedures, especially in vital teeth. Dentin is a hydrated hard tissue in the vital state, where there is an outward flow of dentinal fluid through the dentinal tubules with a positive pulpal pressure, estimated to be approximately 15 cm H_2O.[11] Water presents deleterious effects for adhesive procedures, such as the plasticization of the polymer chains, leading to compromised mechanical properties and hydrolytic degradation of resin and collagen fibrils.[12-14] Several studies have evaluated the microtensile bond strength (μTBS) using simulated pulpal pressure (SPP) during adhesive procedures.[15-20] These studies have demonstrated that the presence of positive pulpal pressure can decrease μTBS and dentin sealing ability.[16,21,22]

The immediate dentin sealing (IDS) technique has been suggested as an alternative to improve the quality of adhesion for indirect restorative procedures.[23-25] In this technique, dentin is hybridized using either a two-step self-etching or a three-step etch-and-rinse adhesive system immediately after preparation and prior to impression taking. Increased μTBS and reduced postoperative sensitivity have been reported for this technique.[24,26-28]

It is possible that use of the IDS technique can reduce the effects of positive pulpal pressure on resin-dentin interfaces, but it has not been evaluated to date. Thus, the aim of this study was to evaluate the effect of SPP and the IDS technique on the μTBS and nanoleakage of interfaces produced by different luting agents. The null hypotheses tested were: 1) IDS produces no difference in the bond strength and nanoleakage; 2) simulated pulpal pressure does not affect interfaces; and 3) there is no difference in the performance of the different luting materials.

METHODS AND MATERIALS

Four resin luting agents were used in this study: two self-adhesive cements (RelyX Unicem [UC], 3M ESPE, St. Paul, MN, USA; Clearfil SA Luting [SA], Kuraray Medical, Okayama, Japan) and two conventional resin cements—one that uses a two-step etch-and-rinse adhesive (RelyX ARC [RX], 3M ESPE) and one that uses a one-step self-etching adhesive (Panavia F [PF], Kuraray Medical). Luting agents were mixed and placed according to the manufacturers' instructions (Table 1).

Teeth were randomly assigned to four experimental groups according to resin cement and then to four subgroups according to presence of SPP (with or without SPP) and dentin sealing condition (IDS or delayed dentin sealing [DDS]). This study design resulted in 16 experimental groups (n=5) according to luting agent, pulpal pressure, and dentin sealing.

Tooth Preparation

One hundred twelve recently extracted caries-free third molars stored in 0.1% thymol (Symrise GmbH, Holzminden, Germany) solution at 4°C for no longer than three months were used in this study (80 for the μTBS test and 32 for nanoleakage evaluation). Teeth were obtained by protocols that were approved by the University's review board. After disinfection and removal of soft tissues, flat middle depth coronal dentin surfaces were exposed with 600-grit SiC paper (3M of Brazil Ltd, Sumare, Brazil) under running water to create a standardized smear layer. Teeth had their roots removed using a diamond saw (ISOMET, Buehler, Lake Bluff, IL, USA) 2 mm below the cemento-enamel junction. Pulpal tissue was gently removed to prevent damage of the predentin region.

SPP

To simulate pulpal pressure on the dentin surface, each tooth was bonded to a Plexiglass platform ($3 \times 3 \times 0.3$ cm) penetrated by a 1-mm-diameter stainless steel tube and fixed with cyanoacrylate adhesive (Loctite Super Bonder Gel, Henkel, Düsseldorf,

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

Type	Manufacturers (lot number)	Composition	Application technique
Dual-polymerizing resin cement + two-step etch-and-rinse adhesive	RelyX ARC (N097266) + Adper Single Bond 2 (7MY) (3M ESPE)	Cement: Bis-GMA, TEGDMA polymer, zirconia/silica filler Etchant: 35% H_3PO_4 Adhesive: bis-GMA, HEMA, UDMA, dimethacrylates,ethanol, water, camphorquinone , photoinitiators, polyalkenoic acid copolymer, 5-nm silica particles	a (15 s); b (15 s); c; d; e; i (10 s); mix cement; apply mixture
Dual-polymerizing resin cement + one-step self-etching adhesive	Panavia F (paste A, 00249D; paste B, 0027A) + ED Primer (primer A, 00262A; primer B, 00137A) (Kuraray Medical)	Primer A: HEMA, 10-MDP, 5-NMSA, water, accelerator Primer B: 5-NMSA, accelerator, water, sodium benzene sulphinate Paste A: 10-MDP, silanated silica, hydrophobic aromatic and aliphatic dimethacrylate, hydrophilic dimethacrylate photoinitiator, dibenzoyl peroxide Paste B: silanated barium glass, sodium fluoride, sodium aromatic sulfinate, dimethacrylate monomer, BPO	h (A + B) (leave undisturbed for 60 s); mix cement; apply mixture; i (40 s)
Dual-polymerizing self-adhesive resin cement	RelyX Unicem (366321) (3M ESPE)	Base: glass fiber, methacrylated phosphoric acid esters, dimethacrylates, silanated sílica, sodium persulfate Catalyst: glass fiber, dimethacrylates, silanated sílica, p-toluene sodium sulfate, calcium hydroxide	Mix cement; apply mixture; i (40 s)
Dual-polymerizing self-adhesive resin cement	Clearfil SA luting (00081A) (Kuraray Medical)	Paste A: MDP, Bis-GMA, TEGDMA, hydrophobic aromatic dimethacrylate DL-camphorquinone, benzoyl peroxide, initiator, silanated barium glass filler, silanated colloidal silica Paste B: Bis-GMA, hydrophobic aromatic dimethacrylate, hydrophobic aliphatic dimethacrylate, accelerators, pigments, surface-treated sodium fluoride, silanated barium glass filler, silanated colloidal silica	Automix cement; apply mixture i (40 s)
Two-step self-etching adhesive system	Clearfil SE Bond (00788A) (Kuraray Medical)	Primer: MDP, HEMA, hydrophilic dimethacrylate, DL-camphorquinone, *N,N*-diethanol p-toluidine, water Bond: MDP, Bis-GMA, HEMA, hydrophobic dimethacrylate, DL-camphorquinone, *N,N*-diethanol p-toluidine, silanated colloidal silica Paste A and paste B: as described above	f (20 s); e; g; i (10 s)

Application technique: a, acid etch; b, rinse surface; c, dry with cotton pellet; d, apply one-bottle adhesive; e, gently air dry; f, apply primer; g, apply adhesive; h, apply mixture; i, light polymerize; j, autopolymerize. Abbreviations: Bis-GMA, bisphenol A diglycidyl ether methacrylate; HEMA, 2-hydroxyethyl methacrylate; 10-MDP, 10-methacryloxydecyl dihydrogen phosphate; 4-META, 4-methacryloyloxyethyl trimellitate anhydride; 5-NMSA, N-methacryloxyl-5-aminosalicylic acid; TEGDMA, triethylene glycol dimethacrylate; UDMA, urethane dimethacrylate.

Germany). The pulp chamber was filled with distilled water via a polyethylene tube connected to a syringe barrel with 10 mL distilled water and suspended 15 cm from the tooth crown. Thus, each specimen was connected to a hydraulic pressure device that delivered 15 cm of water pressure.[11,16] For the group not subjected to SPP, the apparatus was not assembled.

IDS

For IDS, the two-step self-etching adhesive Clearfil SE Bond was applied according to the manufacturer's instructions. After initial light curing (Optilux 501, Demetron/Kerr, Danbury, CT, USA) for 10 seconds, the adhesive layer was covered with a layer of glycerin gel (KY gel, Johnson & Johnson, Sao Paulo, Brazil) and light polymerized for 20 seconds to avoid the oxygen inhibition layer. After rinsing off the gel, the teeth were stored in water at 37°C for seven days before the luting procedures. Following this storage time period, sealed dentin was cleaned by airborne particle abrasion with 50-μm aluminum oxide particles (Asfer Indústria Química Ltda, São Caetano do Sul, Sao Paulo, Brazil).

The teeth submitted to IDS and SPP were kept under hydrostatic pressure for eight days, starting 24 hours before IDS.

Other groups, without IDS and with SPP, were kept under hydrostatic pressure for 48 hours, starting 24 hours before the luting procedure.

Luting Procedures for μTBS

Four-millimeter-thick composite resin discs 12 mm in diameter were prepared by layering 2-mm-thick

increments of a microhybrid composite resin (Filtek Z250, shade A1, 3M ESPE) into a silicone mold. Each increment was light activated for 40 seconds with the Optilux 501 (700 mW/mm^2) halogen light curing unit (LCU). One side of the composite resin discs was abraded with 600-grit SiC paper under water cooling to create a flat surface with standardized roughness. The composite surface was airborne particle abraded with 50-μm aluminum oxide particles for 10 seconds. Before luting procedures were performed, the composite resin discs were ultrasonically cleaned in distilled water for 10 minutes, rinsed with running water, air dried, and silanated (RelyX Ceramic Primer, 3M ESPE).

Excess water was removed with cotton pellets. Care was taken not to dehydrate dentin surfaces. In all groups (with or without IDS and SPP), the adhesive system (when necessary) and luting agents were applied according to the manufacturer's instructions. Composite resin discs were pressed on the cement using digital pressure, which was sustained until light curing was performed from the buccal and lingual sides. The cementation procedures were randomly processed. Specimens were light activated for 40 seconds from the buccal, lingual, and occlusal directions. Bonded specimens were stored in distilled water for 24 hours, and the specimens submitted to SPP were kept under constant pulpal pressure during the same storage time.

Luting Procedures for Nanoleakage Analysis and Scanning Electron Microscopy Evaluation

For each experimental group, two additional teeth were similarly prepared as previously described, with the exception that after luting agents were mixed and applied onto the dentin surfaces, a polyester strip was placed over the luting agent, and a glass slide was used to apply digital pressure while the luting agent was light activated for 40 seconds with the LCU. Afterward, a thin layer of a low-viscosity resin composite (Surefil SDR flow, Dentsply Caulk, Milford, DE, USA) was applied and light activated for 40 seconds. After storage in similar conditions to those described above, teeth were sectioned perpendicular to the adhesive-tooth interface into 0.9-mm-thick slabs using a diamond saw (Isomet 1000, Buehler).

Bonded slabs were coated with two layers of nail varnish applied up to within 1 mm of the bonded interfaces. To rehydrate specimens and avoid desiccation artifacts, they were immersed in distilled water for 20 minutes prior to immersion in the tracer ammoniacal silver nitrate solution for 24 hours. Tooth slabs were placed in the tracer solution in total darkness for 24 hours, rinsed thoroughly in distilled water, and immersed in a photodeveloping solution for eight hours under a fluorescent light to reduce silver ions into metallic silver grains within voids along the interface.

For scanning electron microscopy (SEM) analysis, two slices of each tooth were fixed in Karnovsky's solution and embedded in epoxy resin (Epoxycure, Buehler). Afterward, they were polished with 400-, 600-, 1200-, and 2400-grit SiC paper and 6-, 3-, 1-, and 0.25-μm diamond paste (Arotec, Cotia, Sao Paulo, Brazil). Then, specimens were dehydrated in ascending ethanol series and coated with carbon (MED 010, Balzers Union, Balzers, Liechtenstein). Resin-dentin interfaces were observed with a SEM (LEO 435 VP, LEO Electron Microscopy Ltd, Cambridge, United Kingdom) operated in the backscattered electron mode. After SEM analysis, representative leakage patterns at the cement-dentin interfaces produced by each system were photographed at 500× magnification.

μTBS Evaluation

Twenty-four hours after cementation procedures, the restored teeth were serially sectioned perpendicular to the adhesive-tooth interface into slabs and then the slabs into beams with a cross-sectional bonded area of approximately 1 mm^2 (±0.2 mm^2) using a diamond saw (Isomet 1000, Buehler). Beams were fixed to the grips of a universal testing machine (EZ Test, Shimadzu Corp, Kyoto, Japan) using a cyanoacrylate adhesive (Loctite Super Bonder Gel, Henkel, Düsseldorf, Germany) and tested in tension at a crosshead speed of 1 mm/min until fracture occurred. Maximum tensile load was divided by specimen cross-sectional area, measured with a digital caliper (Mitutoyo Co, Tokyo, Japan) to express results in units of stress (MPa). Five beams were selected from each restored tooth, and the average value for each tooth was used in the calculations.

Failure modes were determined by examination of fractured specimens with SEM (LEO 435 VP, LEO Electron Microscopy Ltd). Specimens were mounted on aluminum stubs and gold-sputter coated (MED 010, BAL-TEC AG, Balzers Union) prior to viewing at different magnifications. Failure modes at the fractured interface were classified into one of four types: CD (cohesive failure in dentin), AD (adhesive failure between hybrid layer and dentin), CC (cohesive failure in the cement), or ADR (adhesive failure between the luting agent and composite

Table 2: Dentin Bond Strength Values in MPa (SD) for the Different Resin Cements and Different Dentin Conditions (Delayed Dentin Sealing or Immediate Dentin Sealing and With Simulated Pulpal Pressure or No Pulpal Pressure)

Product type	Resin cement	Delayed dentin sealing		Immediate dentin sealing	
		No pressure	Hydrostatic pressure	No pressure	Hydrostatic pressure
Two-step etch-and-rinse adhesive/resin cement	RelyX ARC/Single Bond	53.0 (8.6)Aa	34.8 (11.3)Ab	44.8 (3.1)Aa	42.3 (8.8)Aa
One-step self-etching adhesive/resin cement	Panavia F/ED Primer	14.1 (4.6)Ba	7.8 (1.4)Ca	26.2 (15.5)Ba*	22.1 (5.5)Ba*
Self-adhesive cement	Clearfil SA Luting	13.1 (11.1)Ba	14.3 (5.3)BCa	50.5 (5.6)Aa*	33.2 (8.5)ABb*
Self-adhesive cement	RelyX Unicem	14.2 (5.6)Bb	24.2 (2.3)ABa	52.2 (5.4)Aa*	38.7 (8.0)Ab*

Means followed by different uppercase letters (columns), lowercase (rows within each sealing condition), and asterisks (rows within each hydrostatic pressure condition) are significantly different by Tukey test at the 5% confidence level.

resin). Instead of classifying failures as mixed, the area percentage of each type of failure in each specimen was recorded.

Bond strength values were submitted to three-way analysis of variance (ANOVA) considering the factors cement, pulpal pressure, and dentin sealing ($4 \times 2 \times 2$) and Tukey *post hoc* test (α=0.05; SAS for Windows V8, SAS Institute, Cary, NC, USA).

RESULTS

μTBS

Mean μTBS values are presented in Table 2. Three-way ANOVA revealed significant differences for the factors cement (p=0.00001), pulpal pressure (p=0.00084), and dentin sealing (p=0.00001). The double interaction for factors cement × dentin sealing was also significant (p=0.00001). However, the double interactions cement × pulpal pressure (p=0.33868) and pulpal pressure × dentin sealing (p=0.08434) were not significant. The triple interaction cement × pulpal pressure × dentin sealing was also found to be significant (p=0.00064).

Among the four tested groups that were not subjected to SPP or IDS, RX showed significantly higher μTBS than the other materials, whereas PF, SA, and UC presented similar μTBS values when exposed to this same condition (no SPP/DDS). In the presence of SPP and DDS, RX and UC presented the highest μTBS values and were not significantly different. SA self-adhesive cement had intermediate values and did not differ from UC and PF, which presented the lowest μTBS.

Comparing the groups in which DDS was performed (with or without SPP), a significant reduction in μTBS was observed for RX when SPP was applied. The opposite occurred for UC, which presented significantly higher μTBS values in the presence of SPP. However, SPP did not affect the μTBS values of PF and SA.

When IDS was evaluated without SPP, the highest μTBS values were observed for RX, SA, and UC, with no differences among them. PF presented the lowest μTBS compared with the other cements for this condition (IDS, no SPP). When IDS was performed in the presence of SPP, RX presented the highest μTBS values and was not significantly different from SA and UC. PF showed values significantly lower than RX and UC but was not different from SA. When IDS was performed, SPP did not affect μTBS for RX and PF, whereas a significant reduction was observed for SA and UC.

When groups with and without SPP were compared, RX demonstrated no changes in μTBS when IDS or DDS was performed. However, significantly higher μTBS values were recorded for PF, SA, and UC when IDS was performed, either with or without SPP.

Failure mode analysis (Figure 1) showed a prevalence of adhesive failures between resin cement and dentin (sealed or not). The only exception was for RX applied with DDS, which showed a high percentage of cohesive failures in the resin cement. It was observed that failure modes were mainly classified as adhesive (between resin cement and dentin) and cohesive within resin cement. No cohesive failure in dentin or adhesive (between resin cement and indirect composite restoration) was detected. For RX, there was an increase in the percentage of adhesive failures when IDS was performed. For PF, except for the group with IDS and without SPP, where there was a small percentage of cohesive failure within the resin cement, failure mode was 100% adhesive between resin cement and dentin.

Nanoleakage

Figures 2-5 show representative SEMs for the nanoleakage patterns observed for the different tested groups. For all materials tested, whenever IDS was performed, the resin-dentin interfaces

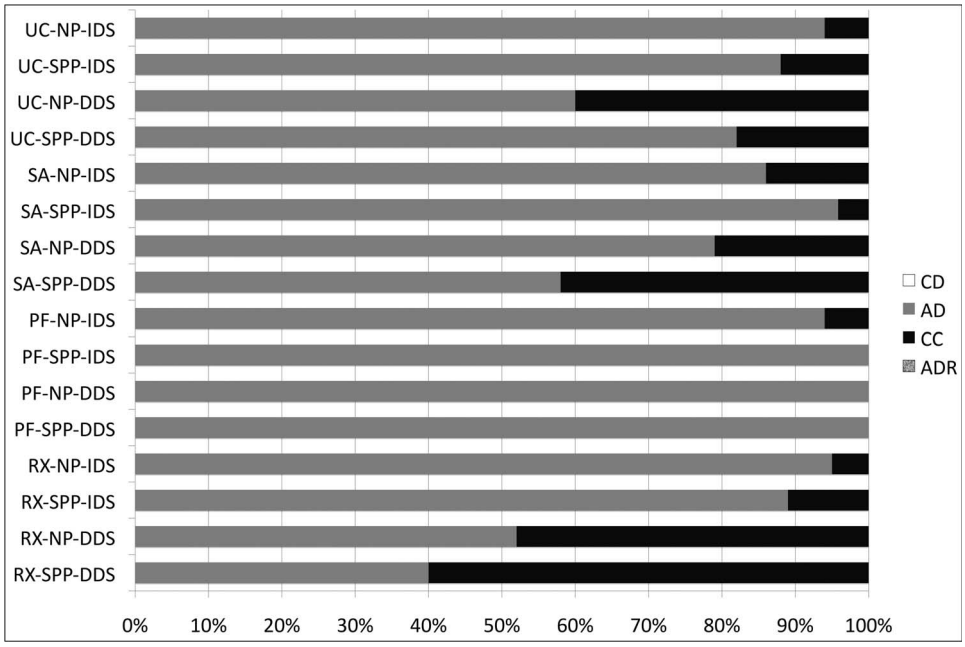

Figure 1. *Distribution of failure modes within groups. CD - cohesive failure within dentin; AD - adhesive failure between hybrid layer and dentin; CC - cohesive failure within resin cement; ADR - adhesive failure between the luting agent and composite resin; NP, no simulated pressure; SPP - simulated pulpal pressure; IDS - immediate dentin sealing; DDS - delayed dentin sealing. UC - Unicem; SA - Clearfil SA Luting; PF - Panavia F; RX - Rely X ARC.*

presented a different characteristic, because there was always the hybrid and adhesive layer produced by Clearfil SE Bond underneath the resin cements.

For groups restored with RX with DDS, an increase in silver deposition was observed in the presence of SPP (Figure 2A,B). However, when IDS was performed, lower silver deposition occurred, which was not affected by the presence of SPP (Figure 2C,D). For PF, silver deposition and gap formation was observed, which increased in the presence of SPP (Figure 3A,B). When IDS was performed, there was a reduction in silver deposition and no gaps were observed (Figure 3C,D).

For the self-adhesive cement SA, little silver deposition was observed when DDS was applied with no SPP (Figure 4A). However, in the presence of SPP,

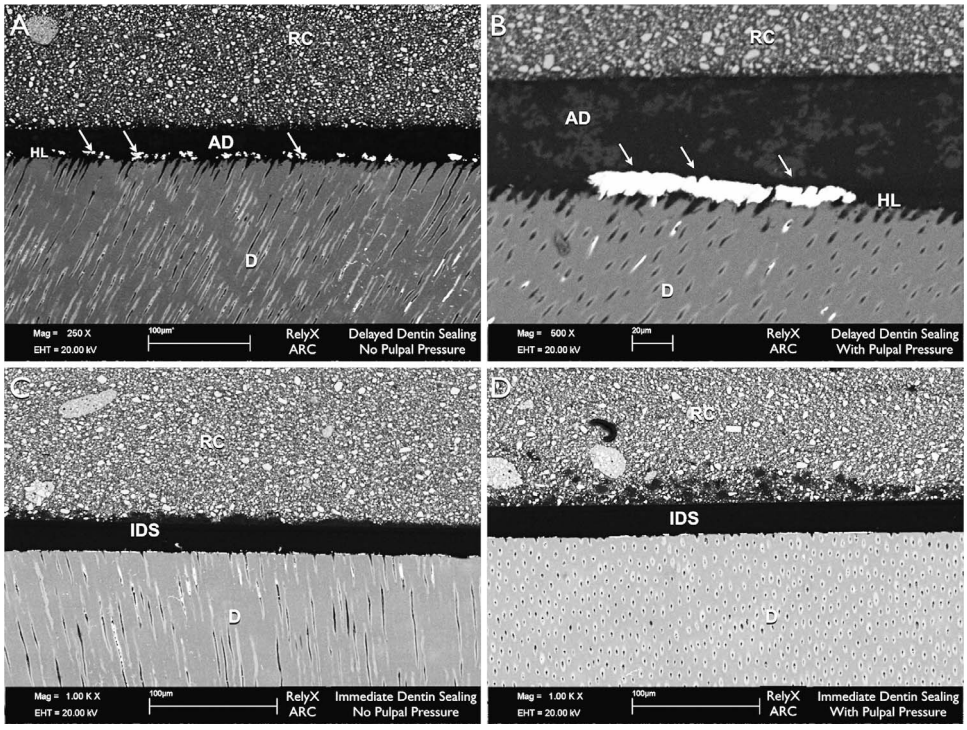

Figure 2. *Representative SEMs of specimens luted with the etch-and-rinse cement RelyX ARC. (A and B): DDS. (A): No simulated pulpal pressure (NPP); silver deposits are indicated by arrows. (B): With SPP, higher amounts of silver deposits were observed. (C and D): IDS. (C): NPP. (D): SPP. RC, resin cement; D, dentin; HL, hybrid layer. Arrows point silver deposits within the interface.*

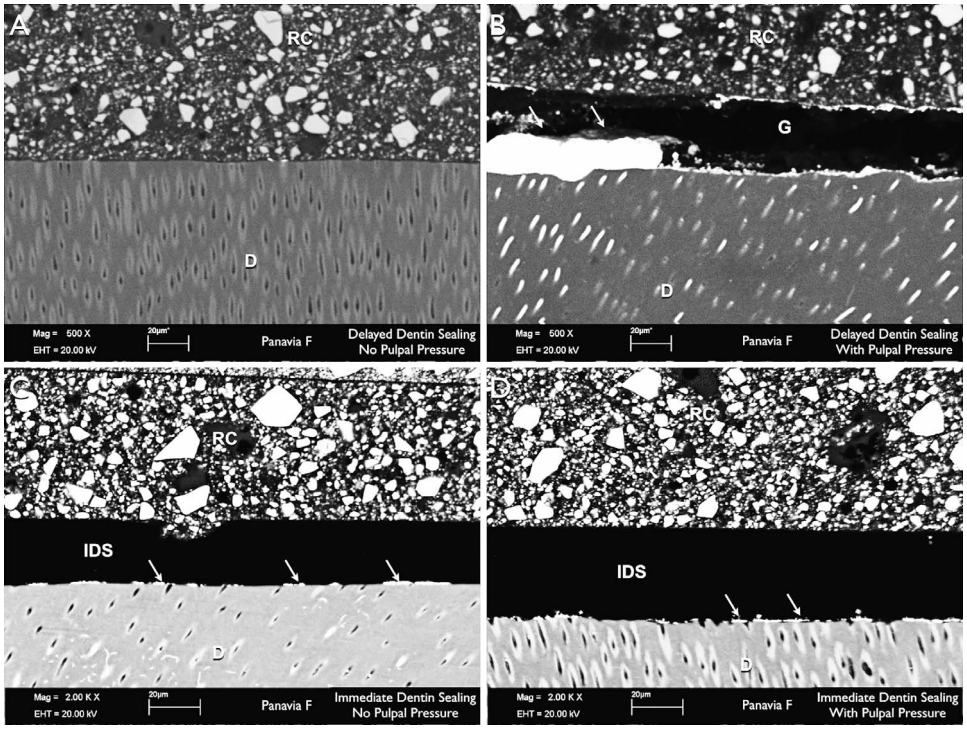

Figure 3. *Representative SEMs of specimens luted with the self-etching cement Panavia F. (A and B): DDS. Silver deposition was observed without (A) and with (B) pulpal pressure. (C and D): IDS. (C): NPP. (D): SPP. The arrows indicate a small amount of silver deposition. DE, dentin.*

silver deposition increased and gaps were present in the interfaces (Figure 4B). When IDS was performed, silver deposition was greatly reduced both in the absence and presence of SPP (Figure 4C,D).

Very little silver deposition was observed for UC self-adhesive cement, even when DDS was applied in the presence of SPP (Figure 5A,B). When IDS was performed, the bonded interface was similar to that

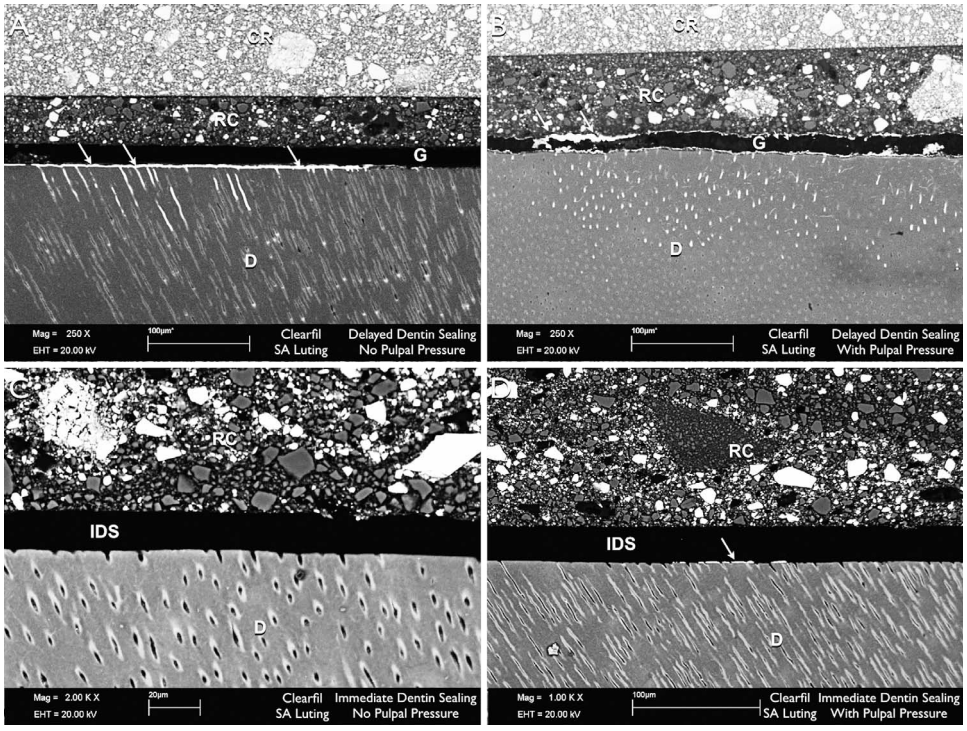

Figure 4. *Representative SEMs of specimens luted with the self-adhesive cement Clearfil SA luting. (A and B): DDS. (A): NPP. (B): Silver deposition was observed when specimens were subjected to SPP. (C and D): IDS. (C): NPP. (D): SPP. The arrows indicate silver deposition. G, gap.*

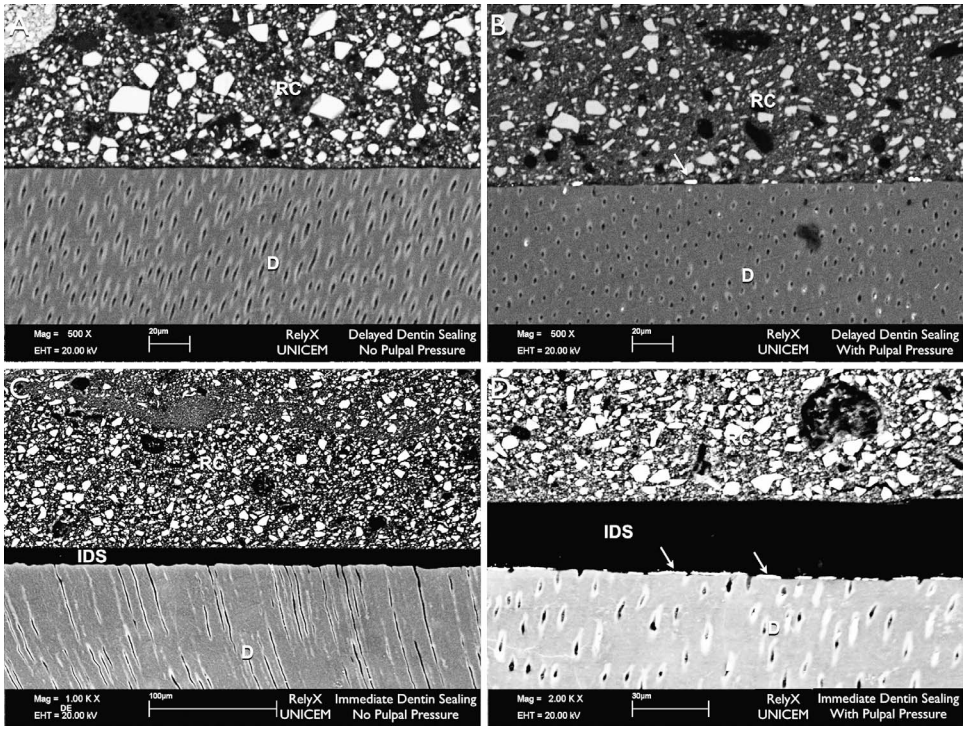

Figure 5. *Representative SEMs of the self-adhesive resin cement RelyX Unicem. (A and B): DDS. (A): NPP. (B): SPP. Almost no silver deposition was observed. (C and D): IDS. (C): NPP. (D): SPP.*

observed for all groups in which IDS was performed (Figure 5C,D).

DISCUSSION

Intrapulpal pressure has been described as one of the factors able to negatively interfere with dentin adhesion, and lower bond strengths have been consistently produced in the presence of positive intrapulpal pressure.[5,8,9,29] The intrapulpal pressure setup used in the present investigation was designed to simulate the *in vivo* bonding condition using water as dentinal fluid. However, besides water, dentinal fluid contains electrolytes, proteins, and enzymes that can provide a greater challenge to the resin-dentin interfaces produced *in vivo*.[29] The sensitivity of some materials to regional variations in dentin morphology can be responsible for the high standard deviation values observed for some of the tested groups.

Conventional resin cements such as RX that use the phosphoric acid etch-and-rinse technique for dentin pretreatment promote complete smear layer removal. This process increases dentin permeability, allowing a greater flow of dentin fluid within dentinal tubules, due to the presence of positive pulpal pressure.[19,22] In this study, RX exhibited reduced bond strength and increased silver deposition when subjected to SPP and DDS. This result may be related to water diffusion in the hybrid layer, reducing mechanical properties of the polymer matrix.[5,14,30,31]

According to Hiraishi and others,[8] water sorption through the adhesive layer may result in an unstable porous region, making it prone to degradation, rendering this interface a weak point in the presence of pulpal pressure.[32,33] For RX, with SPP, porous regions were observed, suggesting the presence of water channels. Despite the presence of silver deposits, the bond strength values observed for groups restored with the etch-and-rinse system RX were the highest under all studied conditions. Although nanoleakage analysis provides important information on the performance and behavior of resin-dentin interfaces, no correlation between bond strength and nanoleakage has been reported, which may explain these results.[34-37]

Even in the absence of SPP, RX and PF presented a certain amount of nanoleakage. In RX, the nanoleakage pattern produced was probably due to the difficulty in completely eliminating water during the adhesive procedure. In addition to water presence in its composition, a two-step etch-and-rinse adhesive system produces a semipermeable membrane due to its high hydrophilic monomers and solvent concentrations.[22,38]

The self-etching resin cement PF subjected to SPP revealed silver deposition by SEM, suggesting permeability within this system. Although no signif-

icant difference was detected for PF μTBS values when SPP was applied with DDS, a considerable reduction in bond strength was observed compared with the DDS group applied with no SPP. This performance is probably related to a higher hydrophilic monomer concentration in the ED Primer, resulting in a highly permeable layer after curing.[2,38] Previous studies have reported a negative influence of pulpal pressure to Panavia F.[8,32] However, when IDS was performed, bond strength values increased significantly compared with the groups without the IDS, despite the presence of SPP. The Panavia F failure pattern was predominantly adhesive between resin cement and dentin.

The role of water is crucial in self-adhesive luting agents, because it promotes the release of hydrogen ions necessary for smear layer demineralization.[39] It is believed that the change from hydrophilic to hydrophobic after curing, which occurs in UC, enhances the stability of the interface. When SPP is applied, water coming from the dentinal tubules can increase aggressiveness of acidic monomers by improving smear layer dissolution and dentin demineralization.[8] This fact probably contributed to the increase in bond strength observed for UC when applied to dentin submitted to SPP.

Except for RX, IDS resulted in higher bond strength values for all tested materials either with or without SPP. Furthermore, nanoleakage reduction was observed when IDS was performed for most materials, including RX, both in the presence and absence of SPP. Due to its higher demineralization ability, the hybrid layer formed by Clearfil SE Bond ($\approx$0.5 μm) is thicker than that produced by self-adhesive cements.[5,7,13,30] In addition, the presence of a hydrophobic adhesive layer may contribute to greater interface stability.[5,10,13]

The IDS technique has been proposed in an attempt to improve the quality of bonded interfaces in indirect restorations, in which dentin is hybridized with a two-step self-etch or a three-step etch-and-rinse adhesive system after preparation. This procedure contributes to the occurrence of increased bond strength and reduces the dentin sensitivity during the provisional phase.[23,24,26]

Effective adhesion between an immediate dentin sealing layer and resin cement probably occurs due to the presence of unreacted methacrylate groups still present in the adhesive layer. Thus, a copolymerization between fresh resin cement and adhesive previously applied during the sealing may occur. Failure pattern analysis demonstrated that fractures in IDS groups usually occurred between the luting agent and the sealing, suggesting that even in the case of bond disruption, the dentin still remains sealed. Magne and others[26] reported that resin cement/sealed dentin bonding might occur due to the presence of residual free radicals,[40,41] van der Waals interactions (intermolecular forces), and micromechanical retention.

Therefore, results of the present investigation suggest that the use of the IDS technique is effective in promoting greater bond strength values and reduced nanoleakage patterns in indirect restorative procedures, especially in the presence of SPP. Our null hypotheses were rejected, because IDS, pulpal pressure, and material selection significantly affected bonded interfaces.

CONCLUSION

SPP decreased the quality of resin-dentin interfaces produced by the multistep resin cements and did not affect the tested self-adhesive materials. However, the IDS technique improved the quality of interfaces of all tested materials, counteracting the negative effects of SPP for the multistep etch-and rinse system RelyX ARC and increasing bond strength values for the self-etching cement Panavia F and the self-adhesive cements RelyX Unicem and Clearfil SA Luting.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of: Guarulhos University Ethics Committee. The approval code for this study is: (SISNEP/384).

Acknowledgements

The authors thank Dr Elliot Kitajima and Dr Francisco Tanaka (University of São Paulo) for technical support with electron microscopy. This study was supported by Sao Paulo State Research Foundation - FAPESP Grants 2007/06083-4, 2008/07628-7, 2009/16261-2, 2008/05179-0, and 2014/23401-3.

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.

(Accepted 17 June 2015)

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Effect of Tooth-structure Thickness on Light Attenuation and Depth of Cure

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KS Vandewalle

Clinical Relevance

Newer bulk-fill resin composites boast a depth of cure of up to 5 mm, with some manufacturers recommending a trans-tooth photo-polymerization technique. While natural human tooth structure significantly attenuates the irradiance from a dental curing unit, clinically favorable depths of cure can be achieved with the use of trans-tooth curing.

SUMMARY

Newer bulk-fill composites claim a greater depth of cure than conventional resin-based composites. To facilitate complete curing, the manufacturer of SonicFill (Kerr) recommends curing from the occlusal, as well as the buccal and lingual, surfaces of the tooth. The purpose of this study was to quantify the degree of curing light attenuation as it passes through natural tooth structure, and how this attenuation affects the depth of cure of different posterior resin composites. Ten noncarious extracted mandibular third molars were sectioned to produce 5-mm-thick pieces of buccal tooth structure. Sanding 0.5-mm increments from the flattened surface produced 4.5-, 4.0-, 3.5-, 3.0-, 2.5-, 2.0-, and finally 1.5-mm-thick sections. A Bluephase G2 (Ivoclar) curing light with an 8-mm-diameter light guide set on high for 20 seconds was used for measurement of irradiance as it passed through different thicknesses of tooth structure and air. The average irradiance (mW/cm^2) was measured with a MARC-RC Resin Calibrator with a 4-mm-diameter sensor (BlueLight Analytics). To measure depth of cure of a conventional hybrid composite (Herculite Ultra; Kerr) vs a bulk-fill hybrid composite (SonicFill) through varying thicknesses of tooth structure, composites were cured in a 4-mm-

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DOI: 10.2341/15-067-L

diameter × 10.25-mm-long split mold according to International Organization for Standardization 4049. A mean and standard deviation was determined per group. Data were analyzed with a one-way analysis of variance (ANOVA)/Tukey test and two-way ANOVA/Tukey test (α=0.05). One-way ANOVA/Tukey found a significant decrease in irradiance based on thickness of tooth structure or distance through air ($p<0.001$). Two-way ANOVA/Tukey found a significant decrease in depth of cure based on thickness of tooth structure ($p<0.001$) and on composite type ($p<0.001$) with no significant interaction ($p=0.623$). SonicFill had a significantly greater depth of cure than Herculite Ultra.

INTRODUCTION

Light-cured resin-based composites have been around since at least the 1960s, with the first visible light-cured composite placed in 1976.[1] Over the decades, these materials have been refined to improve many of their physical properties, including strength, wear resistance, polishability, and handling. A major limitation of resin-based composites is their relative contraindication in certain posterior restorations, particularly high occlusal load areas, and in cusp replacement. To meet this demand, manufacturers have continued to improve the strength of resin composites by incorporating various sizes and shapes of filler particles, starting with the macrofills, up to today's microfills, nanofills, and nanohybrids.[2] The challenge has been to improve strength while maintaining polishability, handling characteristics, and, while less critical for posterior restorations, relatively good to superior esthetics.

Practitioners desire restorative materials that are easy to use, easy to handle, and polymerize quickly and on demand without sacrificing physical properties. A major limitation of direct posterior composites vs direct amalgam restorations is the technique sensitivity of composite placement. Not only do composites require a dry field, extra steps for enamel and dentin etching, priming, and bonding, but placement of the composite-resin material can be critical. The concern of composite shrinkage, and subsequent cusp deflection, is related to the cavity C-factor and composite placement technique, in either a layered or bulk-fill technique. The maximum incremental thickness has historically been 2 mm. However, restoring deeper preparations with 2-mm increments is time consuming and relatively technique sensitive. Manufacturers have introduced new "bulk-fill" flowable and restorative composites, which reportedly can be cured in increments of 4 mm or greater. Studies evaluating the efficacy of incremental vs bulk filling have been somewhat equivocal, with shrinkage stress and cuspal deflection in some studies but reduced cuspal deflection in others.[3,4] Incremental layering may allow flow during curing with additional free surface area. However, incremental curing allows more maximum polymerization and subsequently more shrinkage stress. Little clinical evidence exists to support one particular composite application method over another.[2] Conventional resin-based composites have moved more toward smaller filler particle size for increased esthetics but higher filler load for strength. In contrast, the newer bulk-fill composites generally require greater translucency to achieve greater depths of cure, thus using lower filler loading with larger particle sizes, resulting in reduced hardness compared with conventional composites.[5,6]

Several bulk-fill composite materials are currently on the market, including the low-viscosity formulations, such as x-tra base (VOCO, Indian Land, SC, USA), SureFil SDR (Dentsply, Milford, DE, USA), Venus Bulk Fill (Heraeus Kulzer, South Bend, IN, USA), and Filtek Bulk Fill Flowable (3M ESPE, St. Paul, MN, USA), as well as high-viscosity formulations such as x-tra-fil (VOCO), Tetric EvoCeram Bulk Fill (Ivoclar Vivadent, Amherst, NY, USA), Filtek Bulk Fill (3M ESPE), and SonicFill (Kerr Corporation, Orange, CA, USA). SonicFill and Filtek Bulk Fill boast a depth of cure of up to 5 mm.[7,8] If the cavity preparation is deeper than 5 mm, the manufacturer suggests placing in two increments. By contrast, Kerr's nanohybrid composite, Herculite Ultra, has a recommended maximum incremental cure of 2 mm.[9]

Unlike most other bulk-fill composite materials that have greater translucency to facilitate penetration of visible light for curing, SonicFill reportedly has translucency similar to that of conventional resin-based composites.[6] The manufacturer suggests a curing time of 20 seconds from the occlusal (or follow curing light manufacturer's instructions) and an additional cure from the buccal and lingual aspects for 10 seconds each.[7] While some light directed from a buccal or lingual direction may directly reach restorative material at the line angles, material at the axial-pulpal line angles will rely on light transmitted through either tooth structure from the buccal or lingual or through several millimeters of composite resin from the occlusal aspect. Although research has been done to characterize the amount of light attenuation through resin-based composites,[10] fiber posts,[11] and glass ceram-

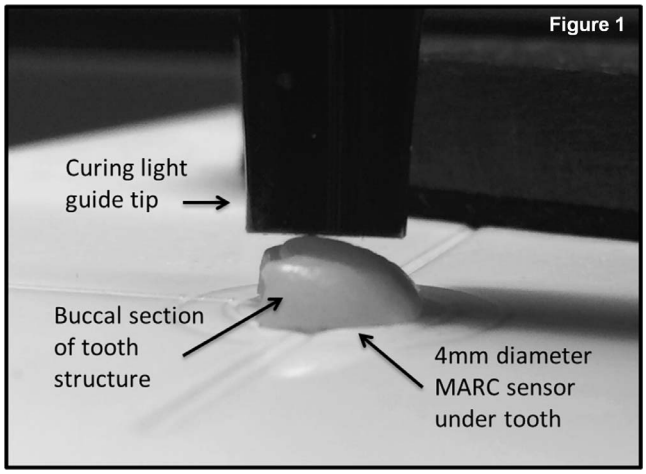


Figure 1. *Measurement of irradiance through tooth structure, with light guide tip directly against maximum convexity of tooth. To measure mean irradiance in air, irradiance was measured at varying distances without tooth structure present, with light guide tip mounted perpendicular and centered on the MARC sensor.*

ics,[12] much less has been done to study light curing through natural tooth structure. In one study by Forsten,[13] two microfilled and one macrofilled composites were exposed with three different curing lights through a 0.8-mm-thick enamel-dentin slab; curing depth was found to be reduced by at least one-third.

The purpose of our study was to test the null hypotheses that natural tooth structure will not significantly attenuate the light from a dental curing unit, and that depth of cure of different resin-based composites will not be affected by curing through tooth structure.

METHODS AND MATERIALS

Sample Preparation

Ten noncarious extracted mandibular third molars were used in this study. The roots were amputated, and the teeth were sectioned to produce 5-mm-thick pieces of buccal tooth structure using an Isomet low-speed saw (Buehler, Lake Bluff, IL, USA). Thickness was measured with a micrometer from the most buccal prominence. The sectioned surfaces were sanded with increasing grits starting with 120 grit, then 320 grit, and finally 600 grit wet/dry sandpaper (Norton, Worcester, MA, USA). After testing, samples were sanded to thinner sections in 0.5-mm increments: 4.5, 4.0, 3.5, 3.0, 2.5, 2.0, and finally 1.5 mm. Orbital sanding paper (120 grit, Diablo, High Point, NC, USA) mounted to the low-speed saw was used for sanding down 0.5-mm increments of tooth, followed by hand sanding with 320- and 600-grit paper. Tooth sections were blotted with a gauze pad to obtain a dry enamel surface and maintain a moist, but not wet, dentin surface, similar to clinical conditions when placing and curing composite-resin restorations.

Irradiance

A Bluephase G2 (Ivoclar Vivadent) curing light with an 8-mm-diameter light guide set on high for a 20-second curing cycle was used for measurement of irradiance from the light as it passed through different thicknesses of tooth structure. The average irradiance (mW/cm^2) from the curing light was measured with a MARC-RC Resin Calibrator (Blue-Light Analytics, Halifax, Nova Scotia, Canada). The most buccal prominence of the tooth samples were centered on the 4-mm-diameter sensor of the MARC-RC Resin Calibrator. The Bluephase G2 curing light was plugged into an electrical outlet to assure a consistent power supply (ie, no battery power under all conditions). The curing light irradiance was measured periodically throughout the study to assure consistent output. The light guide tip was oriented perpendicular and centered on the MARC-RC sensor and directly in contact with the most buccal prominence of the tooth specimen (Figure 1). Irradiance was recorded through each thickness of tooth structure over a 20-second curing cycle using the MARC-RC software. Overall, 10 measurements were made per thickness of tooth structure to include in the statistical analysis. As a control, average irradiance was measured in air over a 20-second curing cycle with the curing light guide tip placed at 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 mm away from the MARC-RC sensor. These distances correspond to the thickness of tooth structure used in the experimental groups and control for reduction in the irradiance due to distance from light guide tip to sensor. Ten measurements per distance were made at each control distance to include in the statistical analysis.

Depth of Cure

To measure depth of cure of a conventional composite (enamel shade A2, Herculite Ultra, Kerr) vs a bulk-fill composite (shade A2, SonicFill, Kerr), composite specimens were cured in a 4-mm-diameter × 10.25-mm-long stainless steel split mold (Sabri Dental Enterprises, Downers Grove, IL, USA). The 4-mm-diameter mold corresponds with the 4-mm-diameter sensor of the MARC-RC Resin Calibrator. A Mylar matrix strip (0.002 inches, Henry Schein, Melville, NY, USA) was placed over the top of the

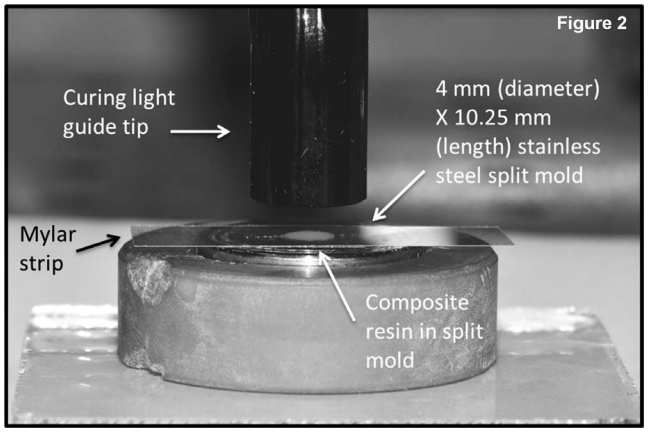


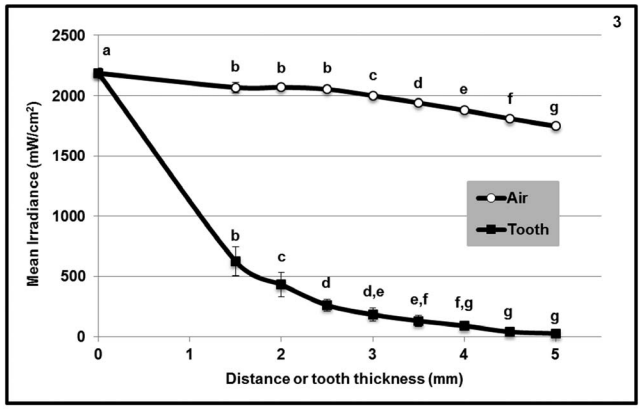
Figure 2. *Measurement of depth of cure at varying distances in air, with light guide tip perpendicular to the composite resin in the stainless steel split mold. To measure depth of cure through tooth structure, buccal sections of tooth at varying thicknesses were placed directly on the Mylar strip, with the light guide tip directly in contact with maximum convexity of tooth.*

split mold, and the curing light was mounted perpendicular to the mold surface. For specimens cured though different thicknesses of tooth structure, the sectioned and sanded surface of the tooth was placed on the split mold with a Mylar strip in between. The curing light guide tip was placed perpendicular to the mold surface and directly against the most buccal prominence. The mold was immediately split apart, and uncured composite was scraped away with a plastic instrument, per International Organization for Standardization (ISO) 4049.[14] The depth of cure was measured with a micrometer (to the nearest 1/100 millimeter), and the value was divided by two to determine depth of cure. For control groups, the curing light was placed directly in contact with the Mylar strip, or at increments of 0.5 mm between 1.5 and 5.0 mm in air, and the composite specimen was cured for 20 seconds on high (Figure 2).

All sectioned tooth specimens were stored at room temperature in 0.5% chloramine-T until use. While testing, specimens were kept at room temperature in distilled water before use. A mean and SD were determined per group.

Data Analysis

One-way analyses of variance (ANOVA) and Tukey post hoc tests were used to examine the effect of tooth thickness or distance through air on mean irradiance. Two-way ANOVA and Tukey post hoc tests were used to examine the effects of composite type and tooth thickness or distance through air on depth of cure.



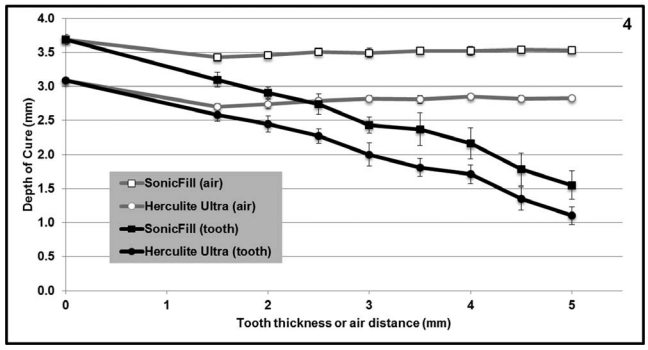


Figure 3. *Mean irradiance (mW/cm^2) vs tooth thickness or distance in air (mm) (no tooth structure between light guide tip and MARC-RC sensor). Error bars represent 1 standard deviation above and below the mean. Increments designated with the same letter are not significantly different ($p>0.05$) per line.*

Figure 4. *Depth of cure (mm) vs tooth thickness or distance in air (mm). Error bars represent 1 standard deviation above and below the mean.*

RESULTS

The one-way ANOVAs with Tukey post-hoc tests found a significant decrease in irradiance based on thickness of tooth structure ($p<0.001$) or on distance through air ($p<0.001$). Figure 3 displays the mean irradiance from the curing light at 0 mm and at half-millimeter increments of distance between 1.5 and 5.0 mm with and without intervening tooth structure. At 0 mm, the mean irradiance from the curing light was 2186 mW/cm^2. A reduction in irradiance was found at increasing distances from the light guide tip to the sensor through air. This reduction ranged from 95% of mean irradiance (2068 mW/cm^2) at 1.5 mm to 80% of mean irradiance (1747 mW/cm^2) at 5.0 mm. The reduction in mean irradiance when light passed through tooth structure was found to range between 28.6% of mean irradiance (625.5 mW/cm^2) through 1.5 mm of tooth structure and 1.2% of mean irradiance (26.7 mW/cm^2) through 5.0 mm of tooth structure.

Two-way ANOVA and Tukey post-hoc tests found significant differences in depth of cure based on thickness of tooth structure ($p<0.001$) and on composite type ($p<0.001$) with no significant interaction ($p=0.623$). Figure 4 displays the mean depth of cure through tooth structure or air. Significant differences in depth of cure were found based on thickness of tooth structure except between the 3.0- and 3.5-mm increment. SonicFill had a significantly greater depth of cure compared with Herculite Ultra. A two-way ANOVA found significant differences in depth of cure based on distance through air ($p<0.001$) and on composite type ($p<0.001$); however, there was a significant interaction ($p=0.003$). Between 1.5 and 5 mm, the depth of cure for both SonicFill and Herculite Ultra cured through air remained relatively unchanged.

DISCUSSION

On the basis of our results, we reject the null hypothesis and accept the alternate hypothesis that there is a significant decrease in the amount of irradiance based on thickness of tooth structure or on distance through air. To our knowledge, this is the first study that has used a specifically designed curing light spectrophotometer, the MARC-RC Resin Calibrator, to quantify the mean irradiance transmitted through natural tooth structure. Figure 3 shows the exponential drop in mean irradiance as light passes through increasing thickness of tooth structure. A previous study using a handheld digital radiometer and 1.0-mm incremental thicknesses of dentin found a similar drop in transmitted light energy, as expressed as a percentage of maximum, ie, that measured without tooth structure between the light guide tip and detector.[15] We also reject the null hypothesis that there is no difference in the depth of cure of two different resin-based composites when cured through different thicknesses of tooth structure or through the equivalent distance in air.

Our rationale for investigating depth of cure when light is passed through tooth structure comes directly from the manufacturer's recommendation for the curing of bulk-fill composites, specifically SonicFill by Kerr, which recommends a 20-second cure from the occlusal, followed by 10-second cures from the buccal and lingual aspects of the tooth.[7] In our study, we chose to use a 20-second curing cycle under all conditions to facilitate direct comparison across experimental and control conditions. Figure 4 shows that, with a single curing cycle of 20 seconds and 0 mm from light guide tip to resin composite, SonicFill had a mean depth of cure of 3.69 ± 0.07 mm, which agrees with other recently published studies using ISO 4049.[16,17] Kerr claims a depth of cure of up to 5 mm for SonicFill, but to achieve this depth of cure, we need to follow the manufacturer's instructions and also cure from buccal and lingual aspects (through tooth structure).

The largest tooth in a buccal-lingual direction on average is the maxillary first molar, with a cervical diameter of ~10.0 mm and a crown diameter of ~11.0 mm.[18] If we consider a class II composite preparation in a maxillary first molar, the gingival/pulpal floor buccal-lingual dimension of one-third the tooth's diameter would be approximately 3.5 mm. This would leave approximately 3.5 mm of tooth structure in both the buccal and lingual directions at the most prominent diameter of the tooth. On the basis of the results from this study, the depth of cure expected for SonicFill with 3.5 mm of tooth structure between the light guide tip and composite would be 2.37 ± 0.24 mm (Figure 4). If cured from both the buccal and lingual directions, a total depth of cure in the buccal-lingual direction of 3.5 mm would be possible. However, as stated above, we set up our *in vitro* conditions such that all curing cycles were for 20 seconds, whereas the manufacturer recommends only 10 seconds. Considering an occlusal depth of cure without intervening tooth structure of 3.69 mm, in addition to the 2.37 mm from both the buccal and lingual directions, a total depth of cure of 5.0 mm as measured from the occlusal surface is possible.

A study by Campodonico and others[19] prepared extracted human teeth with a slot-shaped mesial-occlusal-distal cavity of 4 mm in depth and 4 mm in width, with a mean wall thickness of tooth structure of 2.39 mm. They placed a bulk-fill hybrid composite (x-tra fil, VOCO) and a nanocomposite (Filtek Supreme Plus, 3M ESPE) using incremental and bulk-fill techniques. In addition, specimens with bulk-filled Filtek Supreme Plus were also cured with a trans-tooth illumination technique. They used a simultaneous (two curing units) technique in which they cured for 20 seconds from the buccal and lingual and then 20 seconds from the occlusal. Their results with Filtek Supreme Plus showed that, with the bulk-fill technique, Knoop hardness values decreased beyond a 2-mm depth of cure compared with the incremental placement technique, thus supporting incremental placement with traditional light curing techniques. However, with additional curing using trans-tooth illumination, the authors found that a drop in hardness did not occur until a depth of 3 mm, and the trans-tooth curing technique yielded higher hardness values at a depth of 1.5 mm

compared with restorations placed with the incremental technique. Their study did not test trans-tooth bulk-curing of x-tra fil, a hybrid composite marketed for bulk placement. The instructions for use for x-tra fil call for a 10- to 20-second occlusal curing cycle for an increment of up to 4 mm and also recommend an additional curing cycle from an "oral or vestibular" approach, when accessible.[20]

While Herculite Ultra is not marketed as a bulk-fill composite and the manufacturer states that increments should be placed no more than 2.0 mm in depth,[9] our results show that, whereas Herculite Ultra has a statistically significant reduction in depth of cure compared with SonicFill (Figure 4), the values we found, although less than that of the bulk-fill composite, were greater than 2.0 mm in depth when curing through up to 3.0 mm of tooth structure. It should be noted that achievable depth of cure alone is not the only factor to consider when using a resin-based composite material in either a bulk-fill or incremental-placement technique. The cavity configuration, or C-factor, and polymerization shrinkage of the resin composite can affect the stresses on the cavity preparation walls and the integrity of the resin-tooth interface. With respect to light-cured composite-resins, however, marginal adaptation has not been shown to differ significantly between either incremental placement with occlusal cure, bulk-fill with occlusal cure, or bulk-fill with trans-tooth curing in different hybrid bulk-fill and conventional nanocomposites.[19,21]

We observed a gradual and significant decline in mean irradiance with increasing distance in air (Figure 3), from just over 2000 mW/cm^2 at 1.5 mm to approximately 1750 mW/cm^2 at 5.0 mm. However, the change in depth of cure in either Herculite Ultra or SonicFill did not decrease to the same degree with increasing distance through air (Figure 4). We would expect that with increasing distances from the curing light tip to composite resin, there would be a decrease in the amount of light energy delivered to the composite resin;[22,23] however, the decrease found here is modest. At a 5.0-mm distance in air, 1750 mW/cm^2 mean irradiance delivered over a 20-second curing time gives 35 mJ/cm^2 of total energy. At a 1.5-mm distance in air, 2000 mW/cm^2 mean irradiance delivered over a 20-second curing time gives 40 mJ/cm^2 of total energy. Our data do not show a significant difference in depth of cure through air with either 35 or 40 mJ/cm^2 of total energy delivered to either Herculite Ultra or SonicFill. Perhaps shorter curing cycles and greater distance in air between the curing light tip and the composite resin are needed to demonstrate change in depth of cure. Within our experimental parameters, curing light guide tip to composite-resin distance was not the significant variable, whereas the attenuation of light through tooth structure was significant.

When evaluating depth of cure through air, the two-way ANOVA revealed a statistically significant interaction between distance in air and composite type. It can be noted in Figure 4 that with Herculite Ultra, there is little difference in depth of cure with the light guide tip 1.5 mm away versus curing through 1.5-mm-thick tooth structure. Additionally, there is a statistically significant decline in depth of cure at 1.5 mm in air, and then an increase in depth of cure at 2.0 mm, although mean irradiance was not statistically different at these points (Figure 3). More research is needed to better interpret these more subtle findings and better determine their potential clinical significance.

There are several limitations to the current study. The first is that we used a single dental curing light (Bluephase G2, Ivoclar Vivadent) under all conditions. We also investigated a single bulk-fill composite (SonicFill, Kerr) and a single conventional composite (Herculite Ultra, Kerr), both with a single shade (A2). While the purpose of this study was a preliminary investigation into the affect of tooth structure on trans-tooth curing, we cannot draw any conclusions as to how other bulk-fill or conventional composites would perform under the same conditions, nor how the results would change with other curing lights. Each curing light on the market has a unique light emission spectrum and power. Combined with each light's available light guide tips, different collimation, distal light guide tip diameter, and a unique distribution of emitted light across the light-guide tip,[24] different curing lights would be expected to perform differently.

A second limitation of this *in vitro* study is that our conditions are designed to be as close to ideal as possible. We start with a baseline mean irradiance and depth of cure based on the light guide tip in direct contact with the resin composite material (only a Mylar strip between the two). We also oriented the light guide tip directly perpendicular to the MARC-RC Resin Calibrator sensor, as well as to the top surface of the stainless steel split mold. Such conditions of ideal distance and angulation of the curing light guide tip to the restorative material is rarely possible *in vivo*. Although it is known that distance and angulation are critical for maximum and ideal resin composite photo-polymerization,[23,25]

several factors make this clinically difficult. Interference of matrix bands, oral anatomy, limited opening by the patient, curing light design, and perhaps, most importantly, variable light-curing technique by the dentist or assistant will all affect the quantity of delivered light to the composite resin and in turn affect the total energy delivered.[22] Such clinical limitations require consideration of additional curing time and/or additional curing cycles to achieve ideal photo-polymerization. It has been found that manufacturer recommendations of curing times for photo-initiated composite resins are typically not adequate.[26] Therefore, in our study, it is fair to compare our ideal conditions and results with the manufacturer's recommendations, bearing in mind that clinical conditions will necessitate adjustments compared with more ideal *in vitro* studies and manufacturer recommendations.

A final significant limitation of our study is that we used a single approach to measure depth of cure, the uncured composite resin scrape test.[14] Although the reduction in mean irradiance was very significant with increasing tooth thickness (Figure 3), the reduction in depth of cure, by our measure, was not as dramatic (Figure 4). It is possible that while the scraped thickness test suggests the depth at which an adequate depth of cure has occurred, these data do not quantify the physical properties of the cured composite resin at different depths. However, it has been shown previously that the scraped composite test is well correlated with more sophisticated flexural strength tests.[26] In contrast, another study evaluated Knoop hardness values in composite resin restorative materials placed in bulk with a trans-tooth light curing approach vs incremental placement and cured from the occlusal only and found that hardness values were lower in the bulk-filled samples.[27]

CONCLUSIONS

Within the limits of this study, these data show a significant decrease in mean irradiance and composite resin depth of cure when curing through natural tooth structure compared with curing through air. Although the decrease in mean irradiance through the tooth structure was significant, the corresponding decrease in depth of cure in both a bulk-fill composite (SonicFill) and a conventional hybrid composite (Herculite Ultra) were affected to a much lower degree. Our data indicate that trans-tooth curing of both bulk-fill and conventional composites may aid in the polymerization of resin within deeper areas of the tooth, resulting in a greater depth of cure in both composite types. Further studies are needed to investigate the mechanical properties of resin composite that is cured with a trans-tooth technique.

Regulatory Statement

This study was conducted in accordance with all the provisions of the human subjects oversight committee guidelines and policies of Wilford Hall Ambulatory Surgical Center / Institutional Review Board in Lackland, Texas. The approval code issued for this study is FWH20140106N (406289-1).

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.

(Accepted 30 April 2015)

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The Effect of Resin-modified Glass-ionomer Cement Base and Bulk-fill Resin Composite on Cuspal Deformation

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MF Burrow

Clinical Relevance

Comparing resin composite restorations with flowable resin composite base, resin-modified glass-ionomer cement base, and no base indicates no difference in reducing cuspal movement. Reducing the stresses from polymerization shrinkage is multifactorial.

SUMMARY

Objectives: This study investigated cuspal deformation in teeth restored with different types of adhesive materials with and without a base.

Methods: Mesio-occluso-distal slot cavities of moderately large dimension were prepared on extracted maxillary premolars (n=24). Teeth were assigned to one of four groups and restored with either a sonic-activated bulk-fill resin composite (RC) (SonicFill), or a conventional nanohybrid RC (Herculite Ultra). The base materials used were a flowable nanofilled RC (Premise Flowable) and a high-viscosity resin-modified glass-ionomer cement (RMGIC) (Riva Light-Cure HV). Cuspal deflection was measured with two direct current differential transformers, each contacting a buccal and palatal cusp. Cuspal movements were recorded during and after restoration placement. Data for the buccal and palatal cusp deflections were combined to give the net cuspal deflection.

Results: Data varied widely. All teeth experienced net inward cuspal movement. No statistically significant differences in cuspal deflection were found among the four test groups.

Conclusions: The use of a flowable RC or an RMGIC in closed-laminate restorations produced the same degree of cuspal movement as restorations filled with only a conventional nanohybrid or bulk-fill RC.

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DOI: 10.2341/14-331-L

INTRODUCTION

Stresses from polymerization shrinkage are an inherent drawback of resin composite (RC).[1] These stresses, if sufficiently large, can negatively affect the bond integrity, forming gaps between the cavity surface and restoration[2] and leading to ingress of bacteria. Microleakage, tooth sensitivity, and recurrent caries are potential sequelae. Although the strength of the bonded interface may sustain the stresses from polymerization shrinkage, the bonded restoration can pull on the bonded tooth structure, causing tooth movement and therefore deformation.[3] Layering techniques and light-curing unit parameters have been variables used to investigate the factors that influence polymerization shrinkage stresses.[4,5] For instance, incremental layering of RC with a thickness <2 mm aims to minimize overall shrinkage stress on the bond, which is related to the configuration factor.[3] In addition, polymerization stress is not only affected by polymerization shrinkage and the rate of polymerization[6] but also by the elastic modulus of the material.[7] It has been widely discussed[3,8,9] that lining a cavity with a flexible, low elastic modulus layer of restorative material can absorb the stresses from the polymerization of the overlying conventional RC, thereby reducing the stresses on the interfacial bond.

Restorative materials that possess low elastic moduli, such as resin-modified glass-ionomer cement (RMGIC) or conventional glass-ionomer cement (GIC) have been indicated for use as a lining or base material. The elastic moduli of GIC-based materials have been found to be generally less than that of RC.[10] In RMGIC/GIC-RC laminate restorations, the presence of an intermediate layer of a GIC-based material could lead to less stress on the tooth-restoration interface from polymerization. In heavily restored teeth, this may potentially result in less tooth deformation. Limited literature exists regarding the degree of cuspal flexure from polymerization shrinkage in RMGIC/GIC-RC laminate restorations.[11-13] Restoring endodontically treated teeth with a GIC base has been shown to reduce cuspal strain.[13] Flowable RC (FRC) has also been suggested to act as a stress-absorbing layer and is recommended as a lining or base material.[8,14] Cara and others[15] reported a reduction in cuspal deflection with the use of a FRC liner. De Munck and others[16] found no benefit when an additional layer of elastic FRC was placed between the adhesive and RC. Varied study designs and lack of standardization makes it difficult to achieve a general consensus regarding the effects of FRC and RMGIC/GIC liners on the remaining tooth structure. In addition, no published studies to date have compared a GIC-based lining to FRC lining in RC restorations.

Bulk-fill RCs are materials designed to allow light-curing of RC in bulk, rather than in 2-mm increments. It has been demonstrated that restoring cavities with RC in increments rather than in bulk produces less overall volumetric shrinkage.[4,17,18] In moderately sized restorations, the shrinkage could lead to marked cuspal deformation with microfractures or loss of bond integrity between the adhesive and tooth or RC. There are also concerns that light-curing in bulk, up to 4-mm thick, will lead to insufficient light-curing due to light attenuation at the deepest part of the restoration. If the RC is not polymerized completely, it will potentially lead to resin adhesive degradation and hydrolysis and will affect the physical properties of the restoration.

Recently, a bulk-fill material was introduced (SonicFill, Kerr Corporation, Orange, CA, USA) specifically for the complete restoration of posterior teeth up to a 5-mm depth. This type of bulk-fill RC is extruded into the cavity while connected to a specific handpiece that is claimed to emit sonic energy. According to the manufacturer (Kerr Corporation), the sonic energy causes the viscosity of the composite to reduce and flow into the cavity. The material then returns to its viscous nature before light polymerization. No studies have examined the polymerization effects of this thixotropic bulk-filling material on cuspal movement.

This study aimed to investigate cuspal deformation in teeth restored with different types of adhesive materials with and without a base. Slot mesio-occluso-distal (MOD) cavities were prepared. A sonic-activated bulk-fill RC and conventional RC were used. The base materials used were an FRC or RMGIC.

The null hypotheses tested were that there would be no differences in cuspal deformation in the restoration of approximal cavities with, first, a conventional nanohybrid resin composite or a bulk-fill nanohybrid resin composite, and, second, the presence or absence of low elastic modulus base materials.

METHODS AND MATERIALS

Tooth Selection and Cavity Preparation

Twenty-four intact maxillary premolars extracted for orthodontic reasons were collected. The teeth

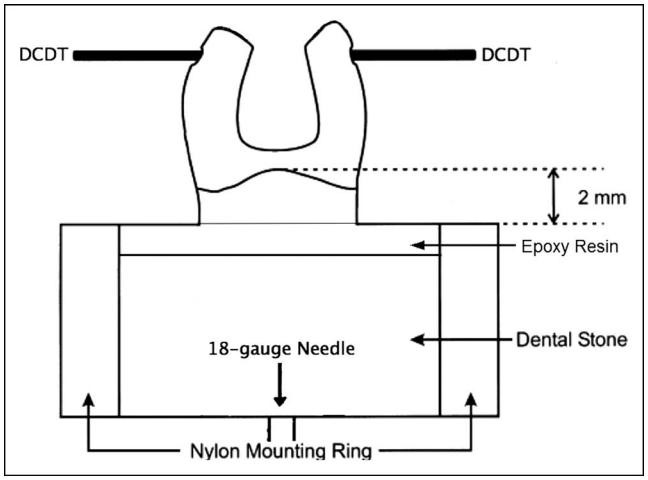

Figure 1. *Diagram of the experimental setup of the prepared tooth mounted in the testing apparatus, with a DCDT probe contacting each cusp to measure cuspal displacement.*

were initially stored in 1% chloramine T solution and then kept in distilled water at 4°C until use. All teeth were used within six months of extraction. Consent was obtained from patients to retain and use their teeth for research under a protocol approved by the appropriate ethics committee. Teeth were sectioned 3 mm below the cementoenamel junction (CEJ) with a diamond saw under water coolant (Struers, Ballerup, Denmark). Pulp tissue was removed with a barbed broach, taking care to avoid contacting the walls of the pulp chamber. The teeth crowns were then immersed in 1% sodium hypochlorite (Endosure Hypochlor 1%, Dentalife, Ringwood, Australia) for 10 minutes, then for an additional five minutes in an ultrasonic cleaner (L & R, Kearny, New Jersey, USA). This was followed by a five-minute period in the ultrasonic cleaner with distilled water, rinsing under running distilled water, and drying before mounting.

The sectioned teeth were mounted in nylon mounting rings and oriented so that the long axis of each tooth was vertical. Before mounting, each ring was filled with type III dental stone (Yellowstone, Ainsworth, Sydney, Australia), just 4 mm short of the top edge, to allow the remainder of the ring to be filled with epoxy resin (EpoFix, Struers, Ballerup, Denmark) flush with the top of the ring. Epoxy resin was left for 24 hours to completely set before a hole was drilled into the center of the set stone to allow placement of an 18-gauge needle. A second epoxy resin (Araldite Ultra Clear, Sellys, Padstow, Australia) was used to seal and cover the sectioned teeth from the set epoxy resin surface to within 2 mm of the CEJ (Figure 1). The maximum buccolingual width of each tooth was measured with a digital micrometer (Bocchi, Pontoglio, Italy) accurate to 0.001 mm. These measurements were used with digital images of each tooth to determine the intercuspal widths (ICWs) using image analysis software (Image J 1.46r, National Institutes of Health, Bethesda, MD, USA). The ICWs were used to equally distribute specimens into four groups of six teeth (Table 1). One-way analysis of variance (ANOVA) using Fisher's least significant difference (LSD) test for multiple comparisons (p=0.05) was used to determine if there were statistically significant differences in the mean ICW between the test groups. The mounted teeth were hydrated for 24 hours with distilled water before cavity preparation.

Table 1: *Intercuspal Width (mm) of Premolar Teeth for Each Group (n = 6 for H, HP, and SF; n = 5 for HRLC)*

Group	Mean (SD)
H	5.69 (0.36)
HP	5.56 (0.23)
HRLC	5.67 (0.59)
SF	5.5 (0.53)

Abbreviations: H, Herculite Ultra; HP, Herculite Ultra + Premise Flowable; HRLC, Herculite Ultra + Riva LC HV; SF, SonicFill.

Standardized slot MOD cavities were prepared in the mounted teeth with a high-speed cylindrical diamond bur (837010, 100-120 μm medium grit, Horico, Berlin, Germany) under water coolant (Figure 2). The occlusal isthmus was prepared to half the ICW with an occlusal depth of 3.5 mm from the central fissure and measured with a periodontal probe. All cavity margins were finished in enamel. The buccal and lingual walls of the cavity were prepared parallel with rounded internal angles. A new bur was used after five teeth were prepared.

Restorative Procedures

Details of the materials used are listed in Table 2. There were four groups, including one control, representing four different direct adhesive restorative procedures as follows:

H = Optibond XTR + Herculite Ultra (control)
HP = Optibond XTR + Premise Flowable base + Herculite Ultra
SF = Optibond XTR + SonicFill
HRLC = Riva LC HV base + Optibond XTR + Herculite Ultra

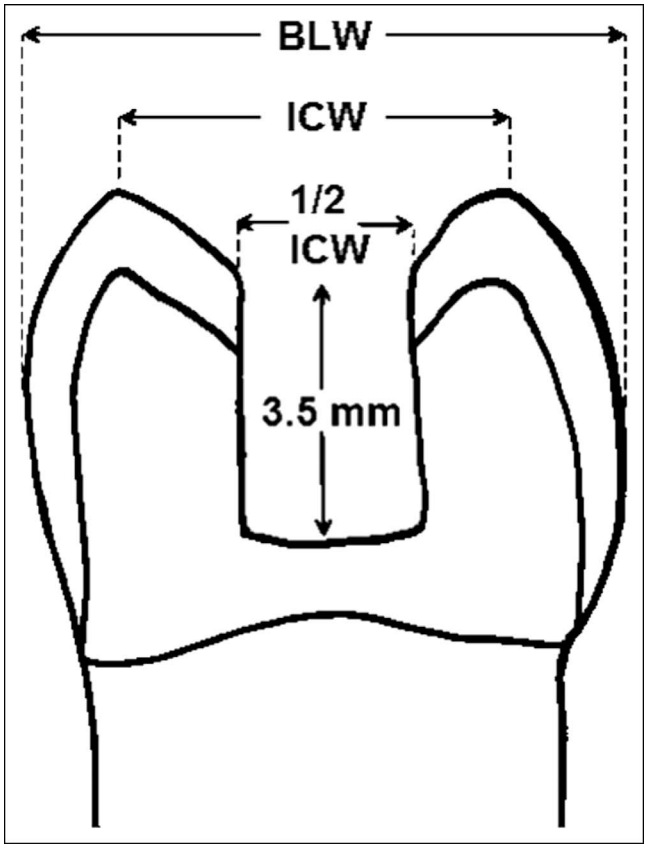

Figure 2. *Cross-sectional diagram of mesio-occluso-distal (MOD) slot cavity prepared in extracted maxillary premolars.*

For the specimens in group HRLC, the dentin was conditioned with 25-30% polyacrylic acid for 10 seconds, followed by rinsing with 5 mL of distilled water. The tooth was placed in the cuspal deflection measurement device while still connected to a distilled water reservoir under 0 kPa hydrostatic pressure. The surface was blot dried with a microbrush before placement of RMGIC (Riva LC HV). A two-step self-etch adhesive, Optibond XTR, was applied in accordance with manufacturer's instructions (Table 2). All groups except for group SF and H were restored with a base, which covered the dentin floor of the cavity. Subsequently, a nanohybrid RC restorative was placed in nine increments with an oblique layering technique. The two proximal walls were restored first (three increments each), followed by three incremental layers within the central occlusal portion. The thickness of each increment was no more than 2 mm to ensure effective light polymerization.[19] For group H, the first increment was also recorded as a pseudo-base for comparison of initial cuspal movement.

Teflon tape 5-mm wide was placed around the circumference of the tooth to act as a matrix band and avoid interfering with the cuspal deflection measuring device. Proximal walls were restored first, followed by the occlusal table. Each increment was cured for 20 seconds using a light-emitting diode (LED) light-curing unit (DemiPlus LED, Kerr Corporation) with an output of 1100 mW/cm^2. A radiometer (Demetron LED radiometer, Kerr Corporation) was used to validate the light intensity immediately before curing the bonding resin, base material, and RC. The light-curing tip was maintained 2 mm above the cusp tips. One operator performed all restorative procedures. Teeth in group SF were restored with SonicFill, a bulk-fill nanohybrid RC. For group SF, after placement and curing of the adhesive, one bulk increment was placed and light-cured for 20 seconds. This was repeated twice more with the light-curing tip placed occlusally toward the proximal region (according to manufacturer's instructions). The total curing time was 60 seconds. Following light-curing of the base and light-curing of the final increment of RC, measurement of cuspal deflection was recorded five minutes after light-curing was completed. This was to allow time for stress relaxation of the polymerized RC.[19] All procedures were performed under 0 kPa hydrostatic pressure. For the groups with an FRC or RMGIC base, the material was placed on the floor of the central part of the MOD slot cavity, approximately 2 mm short of the proximal margins and 1.0-1.2 mm thick. This was measured using a periodontal probe and verified with light-bodied polyvinylsiloxane (PVS) impressions (Elite HD, Zhermack SpA, Rovigo, Italy).

Cuspal Deflection Measurement

Direct current differential transformers (DCDTs, model 7DCDT-050, Hewlett Packard, Rockville, MD, USA) were used to detect linear cuspal displacement. The devices were calibrated to an accuracy of ± 1.0 μm. After the tooth was placed in the cuspal deflection measurement device, the DCDTs were checked for stability before and after recording. The ambient temperature during recordings was 24°C. Two DCDTs were mounted on adjustable arms such that the tip of each rod contacted the buccal or lingual enamel within a shallow depression created 1.0 mm below the cusp tip. The DCDTs were aligned perpendicular to the tooth axis in a buccolingual direction (Figure 1). The DCDTs were placed on either side of the tooth to detect horizontal displacement of the tooth away from the neutral position. Measurements of the two cusps were combined to provide a net inward or outward cuspal movement (in

Table 2: *Materials, Manufacturers, and Chemical Composition*

Type	Material (Manufacturer)	Composition	Batch No.
Resin-modified glass-ionomer cement	Riva Light-Cure HV A3.5 (SDI Limited, Bayswater, Australia)	Liquid: polyacrylic acid, tartaric acid, HEMA; powder: FAS glass	K1201185EG
Dentin conditioner	Riva conditioner (SDI Limited)	Polyacrylic acid	111140
Two-step self-etch adhesive	Optibond XTR (Kerr Corporation, Orange CA, USA)	Primer: pH = 2.4; GPDM, HEMA, MEHQ, CQ, water, ethanol, acetone. Adhesive: pH = 3.3; cross-linking monomers, CQ, inert fillers, barium glass and nano-silica, sodium hexafluorosilicate in ethanol	LD02290
		Instructions: 1) Apply primer to tooth using scrubbing motion for 20 seconds, 2) air thin with medium air pressure for 5 seconds, 3) apply adhesive using light brushing motion for 15 seconds, 4) air thin with medium air pressure and then strong air for at least 5 seconds, 5) light-cure for 10 seconds	LD02291
Flowable nanofilled resin composite	Premise Flowable A3 (Kerr Corporation)	Filler % (wt/vol): 72.5/54.6	4635057
		Filler composition: PPRFs, barium glass, silica; resin composition: bis-EMA, TEGDMA, light-cure initiators, and stabilizers	
Bulk-fill nanohybrid resin composite	SonicFill A3 (Kerr Corporation)	Filler % (wt/vol): 83.5/68	34923
		Filler composition: silica and barium aluminoborosilicate glass; resin composition: TMSPMA, bis-EMA, bisphenol-A-bis-(2-hydroxy-3-methacryloxypropyl) ether, TEGDMA	
Nanohybrid resin composite	Herculite Ultra XRV A3 enamel (Kerr Corporation)	Filler % (wt/vol): 78/57.5	34339
		Filler composition: silica and barium glass, PPRFs, TiO_2, MEHQ, BPO, trimethylolpropane triacrylate, and initiators; resin composition: uncured methacrylate ester monomers	

Abbreviations: bis-EMA, ethoxylated bis-phenol-A-dimethacrylate; BPO, benzoyl peroxide; CQ, camphorquinone; FAS, fluoroaluminosilicate; GPDM, glycerol phosphate dimethacrylate; HEMA, 2-hydroxyethyl methacrylate; HV, high viscosity; MDP, methacryloxydecyl dihydrogen phosphate; MEHQ, 4-methoxyphenol; PPRF, prepolymerized resin fillers; TEGDMA, triethylene glycol dimethacrylate; TiO2, titanium dioxide; TMSPMA, 3-trimethoxysilylpropyl methacrylate.

micrometers). Data from the DCDTs were recorded on a computer using Labview 7.0 software (National Instruments Corp, Austin, TX, USA) then analyzed on a spreadsheet (Microsoft Excel 14.3.6, Microsoft Corporation, Redmond, WA). Data from the two DCDTs were converted from voltages (x) to micrometers (y) via a previously calibrated equation:

$$y = 7.4958x + 10.648$$

Displacement was measured after placement of the base, immediately after final restoration, and then after increasing intrapulpal pressure from 0 kPa to 15 kPa.

Scanning Electron Microscopy (SEM) Examination

Additional specimens (one per group) were prepared, restored, and immersed in distilled water at 37°C for at least 24 hours. Each of the four teeth was sectioned vertically in the mesiodistal plane using a diamond saw (Struers) with water lubricant and then polished using wet 600-, 1200-, 1500-, 2400-, and 4000-grit SiC abrasive papers. All polished tooth sections were etched with 1% HCl for 10 seconds, rinsed with water, immersed in 1% sodium hypochlorite (Endosure Hypochlor 1%, Dentalife) for two minutes and rinsed with 5 mL distilled water. Except for sections with RMGIC, the prepared tooth sections were directly mounted on carbon tape–lined aluminum stubs, gold sputter-coated, and examined using scanning electron microscopy (SEM; Quanta, FEI, Hillsboro, OR, USA) at high vacuum and operating at 2kV. Replicas of the RMGIC tooth sections were produced by taking impressions of the polished sections in PVS; epoxy resin was then poured into the impressions. The epoxy resin replicas were mounted for SEM imaging. The bonding interfaces of the tooth sections were observed.

Table 3: *Mean Cuspal Deflection for Each Restorative Procedure, Mean ± SE*

Group	n	Cuspal deflection (μm)			
		After Base	After Restoration 0kPa	After Restoration 15kPa	Total Deflection
H[a]	6	6.9 (3.84)	12.1 (6.32)	0.93 (0.717)	19.9 (7.87)
HP	6	6.7 (2.41)	13.6 (4.86)	0.38 (0.811)	20.6 (6.86)
HRLC	5	11.7 (5.54)	17.3 (5.16)	0.046 (0.568)	29.0 (7.76)
SF	6	-	23.5 (3.32)	0.86 (0.836)	24.3 (2.76)

Abbreviations: H, Herculite Ultra; HP, Herculite Ultra + Premise Flowable; HRLC, Herculite Ultra + Riva LC HV; SF, SonicFill.
[a] For group H, the first increment was also recorded as a pseudo base for comparison of initial cuspal movement.

Statistical Analysis

Cuspal displacement was calculated by summing the readings of the DCDTs, taking into account the direction of movement of each cusp. The results were normally distributed and analyzed using one-way ANOVA (Minitab 16.2.3, State College, PA, USA), and multiple comparisons were carried out using Fisher's LSD test at a 0.05 level of significance.

RESULTS

Tooth Dimensions

As illustrated in Table 1, the dimensions of the teeth did not vary significantly among the four groups ($p>0.05$).

Base

There was wide variability in the net cuspal movement (Table 3; Figure 3). No statistically significant differences in inward cuspal movement were found between groups HP and HRLC (ie, after an FRC base and an RMGIC base was placed) ($p=0.63$). Additionally, there was no statistically significant difference ($p=0.39$) for specimens with an increment of conventional RC (group H) placed.

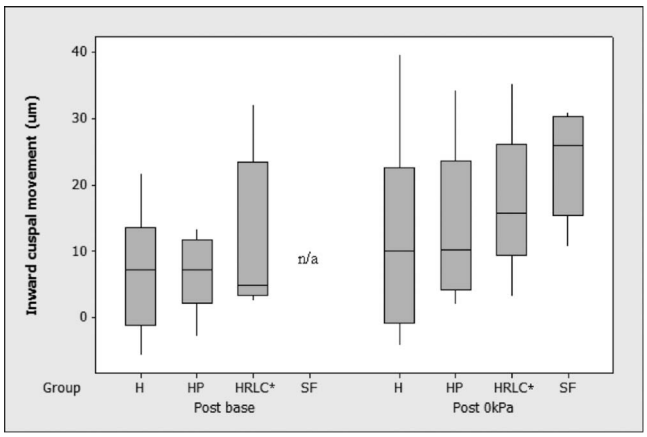

Figure 3. *Box plot displaying the net cuspal deflection for each of the four groups at two stages of the restorative procedure: after base placement (post base) and immediately after restoration with the simulated intrapulpal pressure of 0kPa (post 0kPa). (H = Herculite Ultra; HP = Herculite Ultra + Premise Flowable; HRLC = Herculite Ultra + Riva LC HV; SF = SonicFill) (n = 6 for H, HP, and SF; n = 5 for HRLC).*

Total Deflection

All teeth exhibited inward cuspal movement from baseline (Table 3). There were no statistically significant differences in cuspal deflection values among the four groups immediately after restoration ($p=0.392$). Inward cuspal movement occurred on completion of restoration, which was higher than the cuspal movement immediately after base placement for the HP and HRLC groups and after placement of the first RC increment in group H. Group SF produced the highest and lowest range of inward cuspal movement in contrast to group H (Figure 3). Once the hydrostatic pulpal pressure increased from 0 kPa to 15 kPa, there was minimum deflection and no statistically significant difference in cuspal deflection values among the four groups after restoration (p=0.83). There were no statistically significant differences found in the total deflection among the four groups, ($p=0.772$).

DISCUSSION

Polymerization shrinkage causes stress on the bonding surfaces. In three-surface approximal restorations, both marginal ridges of the tooth are removed, causing the strength of the tooth to be severely compromised.[20] Depending on the polymerization shrinkage and bond strength, placing adhesive restorations in severely compromised teeth will cause cuspal movement. Factors such as elastic modulus of the restorative material, volumetric shrinkage, and rate of polymerization affect the generation of contraction stresses, thereby affecting the durability of the bond.[3,4,21,22] A base or lining material with a low elastic modulus could reduce the degree of overall polymerization shrinkage stress[9] and thereby cause less cuspal movement from the internal compensation of polymerization shrinkage of the overlying RC.

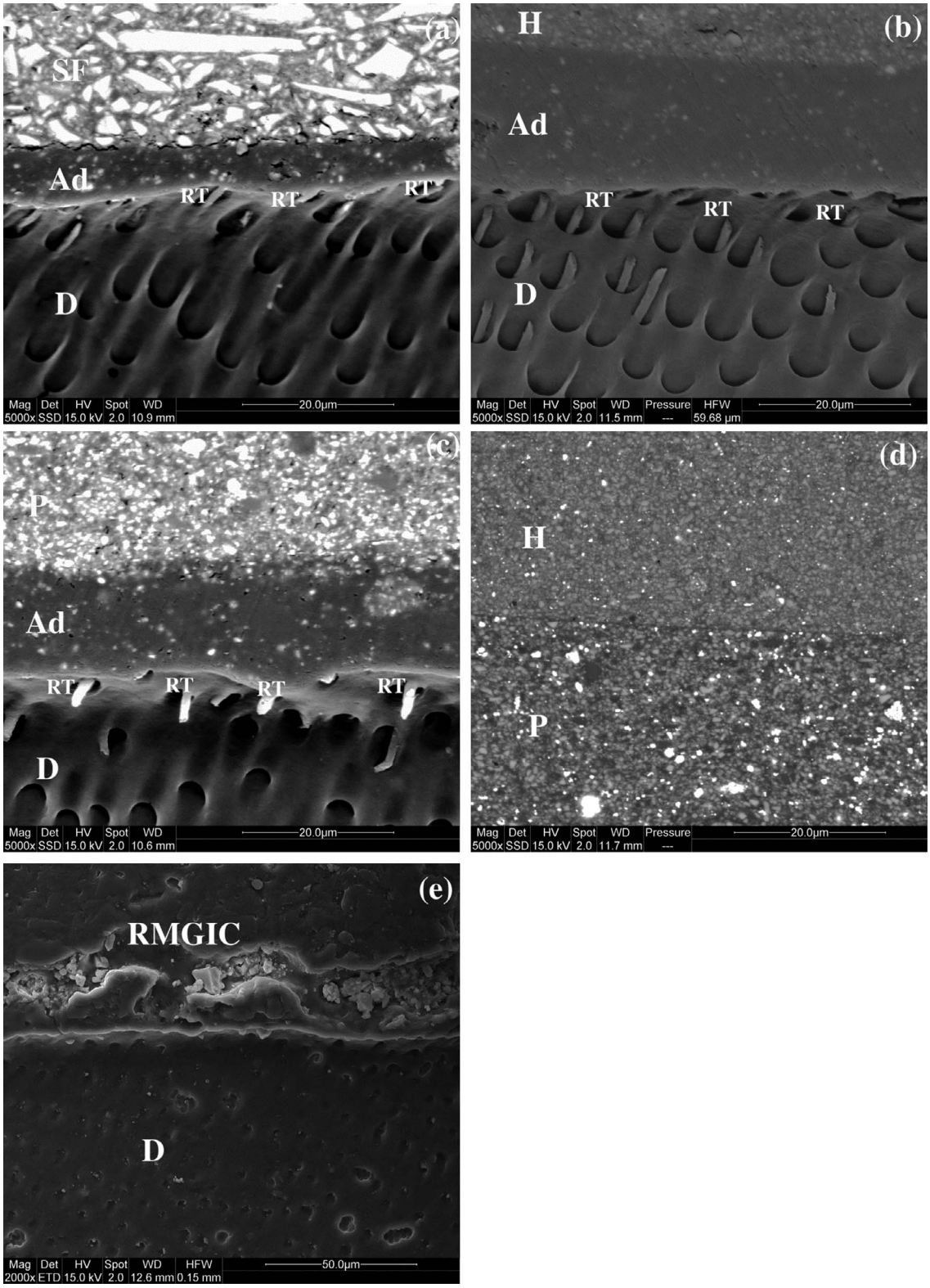

Figure 4. *Representative SEM image of sectioned teeth (a-e). In a-c, resin tags (RT) are evident from the two-step self-etching adhesive, Optibond XTR. Most of these areas showed good adaptation of the adhesive and restorative to the tooth. The filler particles in SonicFill (SF) are irregularly shaped compared with conventional nanohybrid resin composite (H). Selective SEM imaging of the samples from group H displayed adhesive bond layers of 20µm (Ad), as shown in (b). This thickness was more than three times that of the adhesive layers shown in (a) with 6µm thickness, and larger than that in (c). The 15-µm-thick layer may have helped relieve the contraction stresses.[9] In (d), Premise Flowable (P) almost possesses as much filler*

In the current study, an RMGIC base was used as it has a lower elastic modulus than conventional GIC.[23,24] The elastic modulus of RMGIC ranges from 9.0 GPa to 10.8 GPa, which is less than that of high-viscosity GICs and has reported values between 14.4 GPa and 19.3 GPa.[23] The lower elastic modulus is attributed to the resin component in the RMGIC, in particular, the hydrophilic (poly) 2-hydroxyethyl methacrylate.[25] Placing an elastic intermediate layer, such as RMGIC, could reduce stress on the bonded interfaces, thereby reducing cuspal strain and, thus, movement. However, the results failed to show any statistically significant differences in cuspal movement. This could be attributed largely to the wide variability of the data and limited sample size.

RMGICs have also been shown to undergo volumetric contraction after polymerization, which is compensated by a delayed expansion in the presence of water.[26] In a clinical situation, closed-laminate restorations limit absorption of external moisture into the RMGIC base. With the laboratory conditions, the dentin was an internal source of moisture. However, the level of moisture was insufficient to offset the setting contraction of the RMGIC, which explains the comparable degree of inward cuspal movement with teeth restored with FRC base (group HP). However, moisture absorption is gradual as observed from the changes in cuspal movement during the period after restoration. In contrast to the results of this study, a reduction in cuspal deformation with teeth restored with a GIC-based material has been reported.[11] However, these teeth were endodontically treated and subsequently restored with open laminate restorations with a thicker base of 1.5-2 mm. Restoring a portion of the marginal ridge with a thick layer of GIC likely contributed to the reduced cuspal flexure. Alternatively, using FRC could allow relief of developing shrinkage stresses, thereby reducing cuspal strain. However, this was not observed in the current study.

Although FRCs generally have larger shrinkage values than conventional RCs, they have been shown to induce less stress on the interfacial bond.[22] There are limited studies that support this argument[15,22] and studies disputing such claims.[4,9,12] Oliveira and others[12] concluded that the use of liners with low elastic moduli does not compensate for the polymerization shrinkage stress of the overlying RC. They used the FRC Filtek Flow (3M ESPE, St Paul, MN, USA) and the RMGIC Vitrebond (3M ESPE) with a 2.0-mm thickness. However, a three-dimensional photoelastic model was used. Although Filtek Flow possesses an elastic modulus of 13.54 GPa, this was insufficient in relieving the polymerization shrinkage stresses. Braga and others[9] concluded that placing FRCs with elastic moduli of more than 5 GPa is unlikely to produce significant stress relief, even with a thickness of 1.4 mm. This supports the findings of the current study. The results suggest that not all FRCs, such as Premise Flowable, would be indicated for use as an intermediate layer to reduce stress buildup. Premise Flowable has a relatively high flexural modulus of 7.1 GPa (technical information, Kerr Corporation). This likely explains the comparable cuspal deflection values between groups H and HP. It seems that the main clinical benefit of FRC is its handling properties. In addition, representative SEM images (Figure 4) indicated variability in the adhesive thickness between different samples, which may have influenced the results. The thickness of the adhesive ranged from 6 to 20 μm, and this thick resin layer possesses a much lower elastic modulus than the lining materials tested (RMGIC and FRC) in this study.[9] Stresses were likely to have been relieved within this thick adhesive layer.

The higher cuspal deflection values observed in the current study, compared with values reported by other studies,[4,11,19] could be attributed to the high curing light intensity, difficulties in cutting standardized cavity preparations on natural teeth, and possible undetected microfractures in some of the specimens. Moorthy and others[27] reported net cuspal deflection values of approximately 11 μm with the measuring gauge placed 2.5 mm below the cusp tip. As the cuspal wall tapers toward the tip, data recorded with the DVDT probes fixed closer to the tip would detect higher cuspal movement values than if the probes were located more gingivally. It was the authors' intention to create moderate to large cavity preparations, thereby producing noticeable cuspal movements from light polymerization of the restorative materials. In a pilot study, the recording device using DCDTs was shown to be very sensitive (between 5 to 25 μm).

The lowest standard deviation in the SF group would suggest a low technique-sensitive procedure with the bulk-fill system compared with manually placing oblique increments of the conventional RC.

← *volume as Herculite Ultra (H), thus appearing very similar to Herculite Ultra with good adaptation to dentin (D) and Herculite Ultra. In (e), although the irregular surface topography has resulted from the acid preparation, RMGIC appears to have an intimate adaptation to dentin (D).*

Although an increase in filler loading in an RC leads to a reduction in shrinkage, this has been shown to result in higher contraction stresses.[14] SonicFill, an RC designed for bulk-filling, has a filler volume of 68% compared with conventional RC, Herculite Ultra, which has a filler volume of 57.5%. The high filler volume of SonicFill may have offset the volumetric shrinkage from polymerization, as high shrinkage stress was expected from bulk placement of the RC.[17] High shrinkage stress was expected to result in greater cuspal deformation.[4]

According to the manufacturer, the SonicFill material contains modifiers that cause the viscosity to reduce by 87% when a special handpiece is used to dispense the flowable RC into the cavity (technical information, Kerr Corporation). Once in the cavity, the material returns to a viscous material and can then be contoured and light polymerized. It is speculated that reducing the viscosity of SonicFill to allow for efficient restoring procedures is analogous to preheating of RC before incremental placement. Both methods are aimed to improve and simplify adaptation of the restorative material to the cavity by increasing the flowability of the material.[28] Preheating RC before placement in incremental layers does not affect the mechanical properties.[28,29]

There is concern about the efficacy of light polymerization of bulk-fill RCs. Positive correlations between the degree of conversion and microhardness measurements of RC have been observed in previous studies.[30,31] Vickers hardness testing of Sonicfill was done in a pilot study as an indirect method[32] to assess the extent of polymerization in the resin material inside the cavity. If the polymerization is not optimized, this variable would affect the study outcomes. The results from the pilot indicated that there was no difference in the level of polymerization at a curing depth of 4 mm for SonicFill placed in bulk and Herculite Ultra placed in two 2-mm increments. Additionally, another pilot study observed changes in dentin permeability and found that SonicFill provides a comparable seal to an incrementally placed RC, Herculite Ultra. Although these findings suggest that SonicFill can be effectively light polymerized in bulk, the degree of conversion of SonicFill needs further assessment.

The filler and resin composition in restorative materials specifically designed for bulk-filling must account for light attenuation. One approach has been to increase the translucency of bulk-fill restorative materials to enhance the depth of cure. Campodonico and others[33] found no difference in cuspal flexure between the bulk-filling material, X-tra fil (VOCO GmbH, Cuxhaven, Germany), and Filtek Supreme Plus (3M ESPE) a conventional RC. Hardness testing found significantly lower values when Filtek Supreme Plus was light-cured in a 3.5-mm bulk layer, as opposed to X-tra fil.[33] This indicates that X-tra fil can be light polymerized in bulk and did not produce large contraction stresses. However, it has been shown to have a relatively high elastic modulus,[34] which is likely attributed to the high filler volume (70.1% volume). This filler volume is similar to that of SonicFill (68% volume). Moreover, with the findings suggesting adequate light polymerization of the SonicFill restorative material, high contraction would be expected, leading to potentially high levels of stress on the interfacial bond. This could therefore result in high cuspal strain, which contradicts the results of the current study.

Polymerization shrinkage is associated with the polymerization rate and degree of conversion of the restorative material.[21] As the distance of the light-curing tip to the bulk-fill restorative material decreases, the intensity of the polymerizing light is gradually decreased. Hence, in teeth restored with SonicFill, less contraction stress was generated in the deepest portions of the cavity, as the low light intensity in these areas would have allowed some relaxation of the polymer chains. The low intensity allowed a delay in reaching the gel point, thereby encouraging stress relief from the effects of polymerization.[35] There was sufficient light irradiance due to the long duration of light-curing performed (60 seconds for each bulk increment), as recommended by the manufacturer (Kerr Corporation). This would have ensured adequate exposure of the energy density for polymerization.[36] The hypothesis supports the comparable cuspal flexure values between bulk-fill and incrementally placed RC restorations obtained.

CONCLUSION

Within the limitations of this study, the null hypothesis cannot be rejected. The use of flowable RC as a base in adhesive restorations led to cuspal strain, which was not significantly less than that of closed-laminate restorations with an RMGIC base and nonlaminate RC restorations. None of the restorative methods appeared to be more beneficial than the other for reducing cuspal deformation. Reducing the contraction stresses between the adhesive restorative material and tooth substrate is complex and involves the interaction of different

factors, such as the elasticity of the material, polymerization rate, degree of conversion, and hydration conditions. Moreover, the bulk-fill RC appears to induce similar degrees of cuspal movement compared with teeth restored with conventional restorative materials.

Acknowledgements

This investigation was supported by NHMRC grant No. 1039604 and the Australian Dental Research Foundation Inc. The authors would like to thank Kerr Corporation and SDI Limited for the donation of materials, Ms Emily Szycman for SEM imaging, Mr Ilya Zalizniak for technical assistance, and Associate Professor Graham Hepworth for statistical advice. This study was undertaken as part of Dr Khanh Nguyen's thesis, in partial fulfillment of the requirements for the MPhil degree.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of Ethics in Human Research Committee of the University of Melbourne, Australia. The approval code for this study is 23 1136562.

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.

(Accepted 16 June 2015)

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Influence of Light-curing Parameters on Biofilm Development and Flexural Strength of a Silorane-based Composite

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Clinical Relevance

Silorane-based composite is less prone to *Streptococcus mutans* biofilm development compared with a conventional methacrylate-based composite and has an acceptable flexural strength. Lower biofilm development may reduce resin composite superficial stain and recurrent caries, thus improving the longevity of restorations.

SUMMARY

Objectives: The aim of this study was to evaluate the differences in biological and mechanical performances of a silorane-based and a methacrylate-based composite. Another aim was to assess the influence of light-curing time and light-curing intensity on *in vitro* biofilm formation and flexural strength of the two tested composites.

Methods: Experiment 1: 432 specimens obtained from a silorane-based composite and from a standard methacrylate-based composite were divided into six groups and light-cured for 10, 20, 30, 40, 60, or 80 seconds, using one of two light-curing intensities, 400 mW/cm^2 or 800 mW/cm^2. At 24 hours, a monospecific *Streptococcus mutans* biofilm adherent to the surfaces of the samples was obtained. Then, a colorimetric technique (MTT assay) was used to evaluate the adherent viable biomass. Two samples per group were observed using confocal laser scanning microscopy. Analysis of variance (ANOVA) and Tukey tests were used to analyze the results ($p<0.05$). Experiment 2: 192 bar-shaped specimens were obtained and light-cured as in the previous experiment. A three-point bend test using a universal testing machine was performed to obtain flexural strength values. ANOVA and Tukey tests were used to analyze the results ($p<0.05$).

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DOI: 10.2341/14-279-L

Results: In experiment 1, a highly significant difference ($p<0.0001$) in biofilm development was shown between silorane-based and methacrylate-based composites. In fact, the silorane-based composite exhibited better biological performance. Significant differences were also found between the two light-curing intensities ($p<0.018$) and for curing times ($p<0.0001$): silorane-based composite light-cured for 80 seconds at 800 mW/cm^2 light-curing intensity showed the lowest biofilm development. In experiment 2, a significant difference in flexural strength ($p<0.0318$) was only found between the different composites. Nevertheless, both resin composites showed flexural strength values in accordance with International Organization for Standardization guidelines even after 10 seconds of light-curing time.

Conclusions: Silorane-based composite was less prone to biofilm development compared with a methacrylate-based composite. Acceptable flexural strength values for both composites were obtained after 10 seconds of light-curing time.

INTRODUCTION

Thanks to their characteristics,[1] resin-based composites (RBCs) have become the most used materials in restorative dentistry.[2] RBCs are generally cured by light-induced polymerization of monomers. Nevertheless, because complete polymerization of these materials never occurs,[3-6] monomers may leach out of composites.[7-10] Previous data showed that the light-curing time of an RBC is a crucial factor in determining the characteristics of surface colonization.[11] This is a very important aspect as it leads to biofilm development, which is one of the most important factors in caries formation.[12-14] In fact, an imbalance of the oral microbial communities combined with an increase of cariogenic bacteria is considered the first step in primary and secondary caries development.[15-18] *Streptococcus mutans* is the main microorganism responsible for caries lesions, hence influencing restoration success over time.[14,19-21]

Another issue with RBC materials is polymerization shrinkage, which is caused by the conversion of monomer molecules into a polymer network.[22] This process induces stresses into the resin restorations and the surrounding tooth structure, which leads to microfractures and/or blistering and, eventually loss of marginal seal.[23-27] Two strategies have been used to overcome this drawback: lowering the number of reactive sites per volume and using new resins.[28] Increasing the molecular weight of the monomers and the filler load are two methods to reduce the number of reactive sites, but an augmented molecular weight can compromise the handling characteristics of resin composites and increase polymerization stress, whereas an overload of inorganic filler saturates the resin capacity to incorporate its particles.[28]

Since Bowen[29] introduced methacrylate-based chemistry in dentistry in 1965, different alternatives have been studied, some of them by Bowen himself. Research on epoxy resins has led to the development of a new kind of monomer, the siloranes.[30-32] A silorane monomer has a hybrid molecule made of a central siloxane ring to which oxirane structural moieties are attached. The silorane matrix is formed by silorane monomers through a cationic ring-opening polymerization process. The opening of the epoxide rings compensates for the polymerization shrinkage,[30] thus generating a material that possibly overcomes one of the main problems of modern RBCs. Moreover, it has been suggested that the siloxane backbone of siloranes provides hydrophobic characteristics to these restorative materials.[28]

Compared with methacrylate-based restorative materials, silorane-based composites show very low polymerization shrinkage but overall have mixed mechanical performances. The silorane-based material has relatively higher flexural strength/modulus and fracture toughness but lower compressive strength and microhardness than the methacrylate-based composites.[33,34]

So far, no studies have investigated biofilm development and flexural strength of silorane-based composites as a function of their curing parameters. Therefore, the aim of this study was to assess the existence of differences in biological (*S. mutans* biofilm formation) and mechanical (flexural strength) performances of a silorane-based and a methacrylate-based composite. Another aim was to assess the influence of light-curing time and light-curing intensity on *in vitro* biofilm formation and flexural strength of the two tested composites. The tested null hypothesis was that the silorane-based and the methacrylate-based composites would not show differences in *S. mutans* biofilm formation and flexural strength. The second null hypothesis was that light-curing time and light-curing intensity would not have any influence on the two tested materials in terms of *S. mutans* biofilm formation and flexural strength.

Table 1: *Composite Resin Compositions According to Manufacturer*

Composite	Organic matrix	Filler
Filtek Silorane	Silorane (3,4-epoxycyclohexylethylcyclopolymethylsiloxane,bis-3,4-epoxycyclohexylethylphenylmethylsilane)	Silanized quartz, yttrium fluoride
Z250	Bis-GMA, UDMA, Bis-EMA	Zirconia/silica

Abbreviations: Bis-GMA, bisphenol-A-glycidyl methacrylate; UDMA, Urethane dimethacrylate Bis-EMA, bisphenol-A-ethoxy dimethacrylate.

METHODS AND MATERIALS

Two commercially available RBCs, based on either silorane (Filtek Silorane, 3M ESPE, Seefeld, Germany) or methacrylate-based resin chemistry (Filtek Z250, 3M ESPE) were used in this study. The shade used was A3, and the compositions are shown in Table 1. All reagents and multi-well plates used in the present study were obtained from Sigma-Aldrich (St Louis, MO, USA) unless otherwise specified.

Specimen Preparation for the Microbiological Procedures

The wells of a 96-well polystyrene plate were separated from the base of the plate and used as molds to create standardized test disks (6.4mm diameter and 1.5mm thickness). For the preparation of a single RBC test specimen, an excess amount of uncured resin-based composite was placed in a single trimmed well, covered with a Mylar strip to prevent the formation of an oxygen-inhibited layer, and then condensed against a glass plate. The disks were randomly divided into six groups and light-cured for 10, 20, 30, 40, 60, and 80 seconds using a light-curing unit (Spectrum 800, Dentsply International Inc, York, PA, USA). The light-curing unit was set at two light-curing intensities (400 and 800 mW/cm^2), thus generating two subgroups differing in light-curing intensity for each time group. The light-guide end was placed directly in contact with one of the two Mylar strips covering the composite surface. A total of 18 disks were produced for each curing time group and light-curing intensity subgroup. After polymerization the specimens were carefully removed from the wells and checked for visible surface irregularities. No finishing procedure was adopted. The plates were stored in a dark place for 24 hours at 37°C to allow complete polymerization of the disks. Then, 200 μL of sterile phosphate buffered saline (PBS) was placed in each well, and the plates were stored for an additional 7 days to allow for the leaching of most of the residual monomers. To remove the leached monomers, each well was washed twice every day using sterile PBS. Subsequently, 16 disks for each group were transferred to new 96-well polystyrene plates. These plates were then sterilized using a chemiclave with hydrogen peroxide gas plasma technology (Sterrad, ASP, Irvine, CA, USA). By limiting the maximum temperature to 45°C, heat-related damage of the RBC specimens was avoided.

Bacteria

All the culture media were obtained from Becton-Dickinson (BD Diagnostics-Difco, Franklin Lakes, NJ, USA). A pure suspension of *S. mutans* strain ATCC 35668 in brain-heart infusion broth (BHI) was obtained after 12 hours of incubation at 37°C in a 5% supplemented CO_2 environment. Cells were harvested by centrifugation (2200*g*, 19°C, 5 minutes), washed twice with sterile PBS, and resuspended in the same buffer. The cell suspension was subsequently subjected to low-intensity ultrasonic energy in order to disperse bacterial chains, and the optical density (OD) was adjusted to 0.3 OD units at 550 nm (Genesys 10-S, Thermo Spectronic, Rochester, NY, USA), which corresponds to a microbial concentration of 3.65×10^8 cells/mL.

MTT Assay Reagents

A tetrazolium salt (MTT) stock solution was prepared by dissolving 5 mg/mL of thiazolyl blue tetrazolium bromide [3-(4,5-)-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide] in sterile PBS, and a phenazinium salt (PMS) stock solution was prepared by dissolving 0.3 mg/mL of N-methylphenazinium methyl sulphate in sterile PBS. The solutions were stored at 2°C in light-proof vials until the day of the experiment, when a fresh measurement solution was made by mixing 1 mL of MTT stock solution, 1 mL of PMS stock solution, and 8 mL of sterile PBS.

A lysing solution was prepared by dissolving 10% vol/vol of sodium dodecyl sulphate and 50% vol/vol of dimethylformamide in distilled water.

Saliva Collection

Unstimulated saliva from three healthy donors was used in this study according to Guggenheim and others.[35] Saliva was collected in chilled test tubes,

pooled, heated at 60°C for 30 minutes to inactivate endogenous enzymes, and then centrifuged (12.000*g*) for 15 minutes at 4°C. The supernatant was transferred in sterile 10-mL tubes, then stored at −20°C. Immediately before starting the experimental session, saliva was thawed at 37°C for 1 hour. Next, 100 μL of saliva was placed into each well of the specimen-containing sterilized plates, and the plates were incubated for 4 hours at 37°C. Then, the saliva was blotted out and the wells were gently rinsed twice with 200 μL of sterile PBS.

Biofilm Development

In this step, 20 μL of the bacterial suspension in early log phase and 180 μL of sterile BHI were placed in each well. The plates were incubated for 24 hours at 37°C in a 5% supplemented CO_2 environment to allow biofilm development. The culture was then discarded and the wells were carefully washed twice with sterile PBS to remove nonadherent cells.

MTT Assay

Specimen-containing plates were filled with 100 μL of MTT solution for each well; the plates were incubated for 3 hours in a dark place at 37°C: during incubation, microbial redox systems converted the yellow salt to intracellular insoluble purple formazan. Then MTT solution was gently discarded, and the intracellular formazan crystals were dissolved by adding 100 μL of lysing solution to each well and incubating again for 1 hour at room temperature in a dark place. Finally, 90 μL of suspension was taken from each well and its absorbance was measured with a spectrophotometer at 550 nm (Genesys 10-S) and expressed as OD units.

Confocal Laser Scanning Microscopy

Two disks for each experimental group were prepared for confocal laser scanning microscopy (CLSM) analysis. However, because of the number of specimen groups, it was decided to analyze only the 10- and 80-second curing time groups at 400 mW/cm^2 curing intensity. This decision was taken after the MTT results highlighted a major difference in biofilm development between these curing time groups.

After the 24-hour incubation, the biofilm growing on the disks was gently washed with PBS to remove nonadherent cells and stained using the FilmTracer Live/Dead Biofilm Viability Kit for microscopy (Invitrogen Ltd, Paisley, UK). The fluorescence from stained cells adherent to the samples was observed using a CLSM (Leica TCS SP2, Leica Microsystems, Wetzlar, Germany). Four randomly selected image stack sections were recorded for each biofilm specimen. Confocal images were obtained using a dry 20× with numerical aperture (NA) of 0.7 objective and digitalized using the Leica Application Suite Advanced Fluorescence Software (LAS AF, Leica Microsystems) at a resolution of 1024 × 1024 pixels, with a zoom factor of 1.0. For each image stack section an average intensity projection and a three-dimensional (3D) reconstruction were obtained. The average intensity projections were done using ImageJ (National Institutes of Health, Bethesda, MD, USA), and Drishti (Ajay Limaye, Australian National University, CAN, AUS http://sf.anu.edu.au/Vizlab/drishti/) was used for 3D reconstructions.

Specimen Preparation and Flexural Strength Evaluation

A modified procedure from the International Organization for Standardization (ISO) 4049/2009 guidelines was used for the flexural strength evaluation. Briefly, a 2-mm thick polyether strip (Impregum, 3M ESPE AG) was obtained; the strip was then cut to obtain multiple bar-shaped standardized holes with a length of 10 mm and a width of 2 mm. For the preparation of RBC test specimens, an excess amount of uncured RBC was placed in the standardized holes, covered with Mylar strips to prevent the formation of an oxygen-inhibited layer, and then condensed against a glass plate to remove excess material. The glass plate was then removed, and the bars from each tested RBC were randomly divided into six groups and light-cured for 10, 20, 30, 40, 60, or 80 seconds using a light-curing unit (Spectrum 800). The light-curing unit was set at two light-curing intensities (400 or 800 mW/cm^2), thus generating two subgroups differing in the light-curing intensities for each time group. The light-guide end was placed directly in contact with one of the two Mylar strips covering the RBC surface. Eight bars for each light-curing time and light-curing intensity subgroup were produced. After polymerization, the specimens were carefully removed from the strip, checked for visible surface irregularities, and stored in a dark place for 24 hours at 37°C to allow complete polymerization.

After that, the bars were submitted to a three-point bend test with a universal testing machine with a crosshead speed of 1 mm/min. The maximum loads at fracture were obtained and the flexural

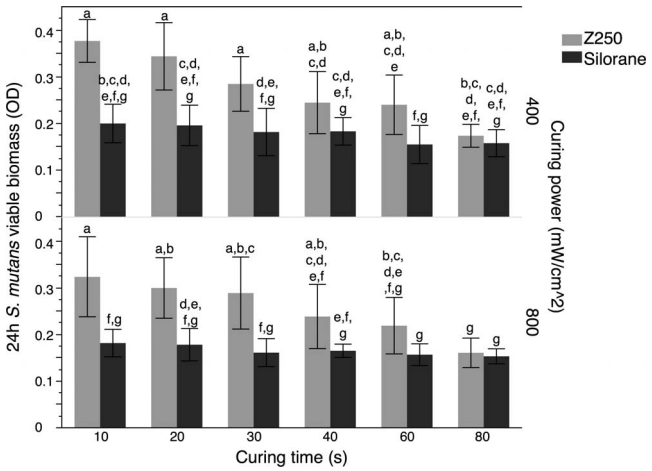

Figure 1. *Biofilm development expressed as OD. Bars represent means and error bars represent standard deviation.*

strength (σ) was calculated in megaPascals (MPa) by using the following formula: $\sigma = 3FL/(2BH^2)$, where F is the maximum load (in Newtons), L is the distance between the supports (in millimeters), B is the width of the specimen (in millimeters), and H is the height (also in millimeters). The formula was solved assuming that the prepared bars had a 2 mm width and a 2 mm thickness, and the custom-made support for the bars had a distance (L) equal to 8 mm.

Statistical Analysis

All statistical analyses were performed using statistical software (JMP 10.0, SAS Institute, Inc, Cary, NC, USA). The OD and flexural data are reported throughout the text as means and standard deviations (SDs) calculated from the natural values.

Data were analyzed by three-way analysis of variance (ANOVA) with balanced data in which light-curing time (six levels, ie, 10, 20, 30, 40, 60, or 80 seconds), light-curing intensity (two levels, ie, 400 or 800 mW/cm^2), and resin composite type (two levels, ie, methacrylate-based or silorane-based composites) were fixed factors. Homogeneity of variances was preliminarily checked using Bartlett's test. Tukey's post hoc test was used to highlight significant differences ($p<0.05$).

RESULTS

Experiment 1

Biofilm development on resin composite discs, expressed as mean OD ± 1 SD, is reported in Figure 1. Methacrylate-based and silorane-based composites showed significantly different biofilm development ($p<0.0001$). Light-curing time ($p<0.0001$) and light-curing intensity ($p<0.0108$) were also found to be significant factors in influencing biofilm development.

For each light-curing time and light-curing intensity group, except 80 seconds, silorane-based composite demonstrated lower biofilm development compared with the methacrylate-based composite. Extended light-curing times and higher light-curing intensities showed a reduction in OD values for both resin composites. However, this phenomenon proved to be significant only for the methacrylate-based resin composite.

The lowest biofilm development was obtained on the surfaces of silorane-based composite light-cured for 80 seconds at 800 mW/cm^2 light-curing intensity, whereas the highest biofilm development was obtained with methacrylate-based composite light-cured for 10 seconds at 400 mW/cm^2 light-curing intensity.

As shown in Figure 2, the methacrylate-based composite light-cured for 80 seconds at 400 mW/cm^2 light-curing intensity and silorane-based composite light-cured for 10 seconds and for 80 seconds at 400 mW/cm^2 light-curing intensity resulted in similar biofilm development with several live (green) and dead (red) *S. mutans* colonies covering the surface of the samples. In contrast, the methacrylate-based composite light-cured for 10 seconds at 400 mW/cm^2 light-curing intensity showed increased biofilm development; most of the surface was covered by live (green) *S. mutans* colonies.

Experiment 2

Flexural strength expressed as mean MPa ± 1 SD is shown in Figure 3**.** Three-way ANOVA did not show any interaction among the considered factors; therefore, analysis was performed for each factor according to a one-way ANOVA model. Flexural strength was only influenced by composite type ($p<0.0318$), and methacrylate-based composite showed higher flexural strength than silorane-based composite. In particular, the best results were obtained by methacrylate-based composite at 80 seconds of light-curing time and 800 mW/cm^2 light-curing intensity; however, they were not significantly different from the other methacrylate-based composite subgroups. Considering silorane-based composite, the best results were obtained for the 80 second 400 mW/cm^2 group. No significant differences between the other silorane-based composite subgroups were observed.

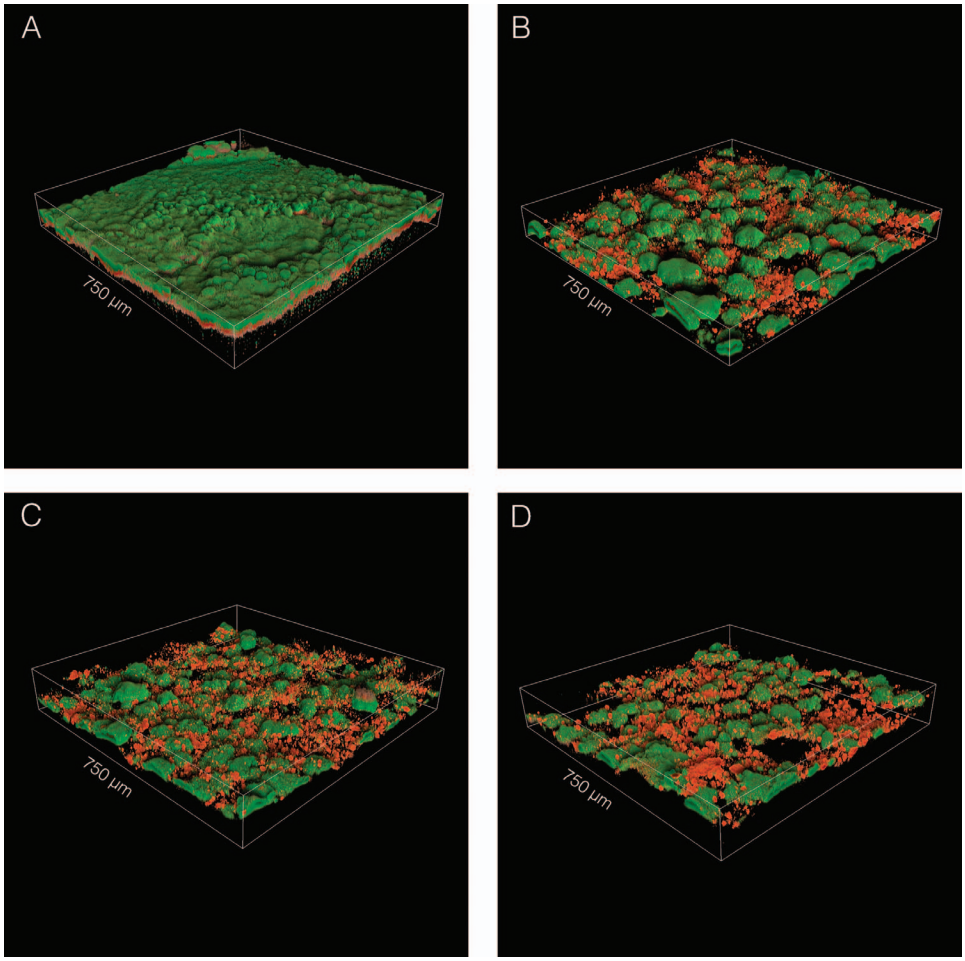

Figure 2. *CLSM 3D reconstructions of the biofilms, which were stained with live/dead stain. Green represents live bacteria and red represents non-viable, dead bacterial cells. Letters refer to the different experimental groups: A = 10 seconds 400 mW/cm^2 methacrylated-based composite, B = 80 seconds 400 mW/cm^2 methacrylated-based composite, C = 10 seconds 400 mW/cm^2 silorane-based composite, D = 80 seconds 400 mW/cm^2 silorane-based composite.*

DISCUSSION

Silorane-based composites were introduced as alternatives to conventional methacrylate-based composites to reduce polymerization shrinkage.[7-10] As bacterial colonization is an important factor for restoration longevity, the evaluation of the biological properties of these alternative resin composites seems to be another important issue to be investigated.

The best way to obtain data on bacterial colonization of the composite surface is to use an *in vitro* experimental model.[36-39] In this study, a Drip-flow reactor was chosen to achieve similar growth conditions for all resin specimens and to keep all the experimental parameters under controlled conditions.[37] Besides the experimental setup of the reactor, different parameters related to the material characteristics need to be considered, in particular the surface roughness and the curing process parameters. The influence of surface roughness on biofilm development was excluded by polymerizing the specimens against a Mylar strip. This technique allowed obtaining specimens with a mean surface roughness of $R_a = 0.06$ µm (data not shown), which is below the 0.2-µm threshold introduced by Bollen and others[40] in the 1990s. The results of this study

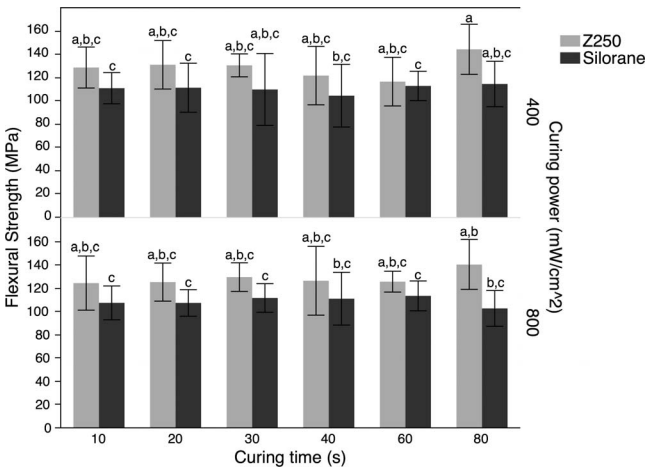

Figure 3. *Flexural strength expressed as megaPascals. Bars represent means and error bars represent standard deviations.*

suggested that R_a values below the specified threshold do not have a significant influence on biofilm development. Regarding the curing process, it has been demonstrated that light-curing time and light-curing intensity deeply influence biofilm development on resin composite surfaces.[11] Consequently it was decided to light-cure the specimens at six different times using two light-curing intensities to obtain data on the influence of these variables. While not of clinical relevance, 80 seconds of light-curing time was used in order to have a group of specimens with the highest degree of conversion possible.

The results of the study allow the rejection of the first null hypothesis because the silorane-based composite surfaces showed a reduction in biofilm development compared with the methacrylate-based composite ($p<0.0001$). In particular, the methacrylate-based resin composite showed a decreasing colonization trend as light-curing time increased, which agrees with the results of a previous study.[11] In contrast, silorane-based composite did not present significantly different values among light-curing times tested. These results allowed us to suppose that physicochemical properties, such as surface roughness and hydrophobicity, could influence material biological behavior. Given that in this study the influence of surface roughness variation could be excluded, it is possible to suggest that silorane had increased hydrophobicity, which makes this material less susceptible to biofilm development.

As for light-curing time, a statistically significant difference ($p<0.018$) in biofilm development was found between the two tested light-curing intensities, but only in methacrylate-based composite. This suggests that for these materials a better biological performance can be achieved by using the highest light-curing intensity tested (800 mW/cm^2).

The results of this study allowed a better comprehension of the tendency of silorane-based composites to develop biofilm, as no other authors have worked on this topic. However, a previous article by Buergers and others[31] demonstrated that the susceptibility of silorane-based composites to bacterial adhesion *in vitro* is lower than for four conventional methacrylate-based composites. The article suggested that the matrix of silorane-based composite, in particular its hydrophobicity, may negatively influence bacterial adhesion,[31] thus confirming our hypothesis. However, saliva was not used, as it was stated that the protocol was kept as simple as possible and that saliva was not the only factor differentiating an *in vitro* study from an *in vivo* study. Yet, another *in vitro* work did not show differences in bacterial adhesion between silorane-based and methacrylate-based composites. Nevertheless, surface roughness values of composites were too nonhomogeneous to easily compare the different groups.[41] Up to now only two *in situ* studies evaluated the biological performances of silorane-based composite. In the first study, Claro-Pereira and others[42] showed similar adhesion values for silorane-based and methacrylate-based composites. Nevertheless, the presence of several variables that were difficult to control and the limited number of subjects involved are weak points of this work. Instead, another *in situ* study evaluated the demineralization of dentin next to multiple restorative materials.[43] Results highlighted a high dentin demineralization associated with silorane-based composite. However, it is difficult to understand how restorative materials without the incorporation of any antibacterial principle can influence dentin demineralization in their proximity. Moreover, in this study, specimens were kept in a prosthesis full of acrylic resin, in which the oral flora is probably very different from the one present on teeth surfaces. With regard to clinical behavior, three clinical trials failed to highlight differences in clinical behavior between methacrylate-based and silorane-based composites.[44-46] In these studies no restoration failed for secondary caries.

Flexural Strength

Flexural strength was investigated to assess the possible influences of light-curing time and light-curing intensity on the mechanical properties of the tested materials but also to investigate if the influence of these parameters was similar on both mechanical and biological performances. According to ISO 4049/2009[47] specifications, dental restorative materials should have flexural strength values above 80 MPa.[47] Both the tested materials meet this standard, even if silorane-based composite values were inferior to the tested methacrylate-based composite, as already pointed out by another study.[34] This conclusion can validate results from a recent clinical trial study in which most of the failures of silorane-based composite were due to fracture.[45] However, as shown by Goracci and others,[48] other conventional composites have flexural strength values similar to those of silorane-based composite.

The ISO specifications also require the length of the specimens to be 21 mm. Although this method may prove useful to provide completely polymerized specimens, it may not provide accurate information regarding the influence of light-curing parameters on the flexural strength of specimens because the tip of the light-curing-source overlaps during polymerization. For this reason, the length of the bars (10 mm) differed from the ISO specifications and was specifically chosen (as equal to the diameter of the fiberglass tip of the light-cure unit) to allow a single-shot polymerization of the specimens.

Results showed that composite type was the only significant factor ($p<0.0318$) and that light-curing time and light-curing intensity did not influence flexural strength. Consequently, the second null hypothesis could also be rejected.

No threshold value indicating a decrease in mechanical properties was identified for any of the tested light-curing times or light-curing intensities. Even if the manufacturer suggested a polymerization time of 20 seconds, testing after 24 hours from the light-curing process showed that maximum flexural strength values were already reached at 10 seconds, independent of the light-curing intensity tested.

CONCLUSIONS

Within the limits of this study, it is possible to conclude that silorane-based composite is less prone to *S. mutans* biofilm development compared with a widely used methacrylate-based composite. Moreover, silorane surface colonization does not seem to be influenced by such factors as light-curing time and light-curing intensity. This may potentially reduce the occurrence of secondary caries, thus improving the longevity of direct composite restorations. Flexural strength was not influenced by light-curing time or light-curing intensity but proved to be significantly higher for the methacrylate-based composite. It is interesting to note the different influence of light-curing parameters on composite mechanical and biological performances.

Regulatory Statement

This study was conducted at the University of Milan.

Conflict of Interest

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

(Accepted 10 March 2015)

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Departments

Faculty Positions

College of Dental Medicine – Illinois [CDMI]: Research Position

Introduction: Midwestern University, initially founded in 1900, is a healthcare educational institution offering professional doctoral degrees in osteopathic medicine, dentistry, pharmacy and other health sciences programs on its two (2) campuses in Illinois and Arizona. The Midwestern University College of Dental Medicine – Illinois (CDMI) is located on our Downers Grove / Illinois Campus with 105 acres boasting innovative facilities, nestled serenely in a wooded setting 25 miles west of downtown Chicago. The total enrolment of the University is over 6,000 students with approximately 550 full time faculty dedicated to providing interprofessional education and the development of leaders in a humanistic and scholarly environment.

Position Description: CDMI is seeking applications for a full-time, tenure-track, faculty position with a focus on Dental Research at the rank of Assistant or Associate Professor. This full-time faculty member will take a lead role in developing and fostering research endeavors among the faculty and students at CDMI. The faculty member will participate in the pre doctoral dental program, mentor faculty members and dental students in their research endeavors, and conduct an active research program. It is expected that the selected candidate will also establish an externally funded research program in educational, basic science or clinical research. The faculty member will be responsible for application of expertise in innovative ways that benefit collaborators from the campus, the community and improves research productivity. All faculty at CDMI are expected to participate in the teaching mission of Midwestern University, to participate in scholarly activity and to demonstrate commitment to service in the larger community.

Qualifications: Candidates must possess a PhD or equivalent degree and an established record of extramural funding with quality scholarly and research productivity in the field of craniofacial, oral, dental, and/or related research. Preference will be given to candidates with a DDS or DMD degree.

The successful candidate will be an individual with excellent communication and interpersonal skills, with the ability to engage newer, clinical faculty in the realm of dental research.

Academic rank and salary will be commensurate with training and experience.

Interested individuals should submit a letter of application and interest, curriculum vitae, and three professional references, plus a one- to two-page summary of a research plan that includes the engagement of clinical faculty, to:

College of Dental Medicine - Illinois
Midwestern University
Attention: Dr. Bruno Jham
555, 31st Street, Auditorium Building 594
Downers Grove, IL 60515
Email: bjham@midwestern.edu

Applications can also be made on-line at www.midwestern.edu.

Midwestern University is an Equal Opportunity/Affirmative Action employer that does not discriminate against an employee or applicant based upon race, color, religion, gender, national origin, disability, or veterans status, in accord with 41 C.F.R. 60-1.4(a), 250.5(a), 300.5(a) and 741.5(a).