Influence of method and period of storage on the microtensile bond strength of indirect composite resin restorations to dentine

Abstract: This study evaluated the influence of the method and period of storage on the adhesive bond strength of indirect composite resin to bovine dentin. Ninety bovine incisors were stored in three different solutions: 0.2% thymol, 10% formalin, and 0.2% sodium azide, during 3 periods of storage: 7 days, 30 days and 6 months, resulting in 9 groups (n = 10). The roots were cut off and the buccal surface was ground with #600-grit silicon carbide paper. The surface was conditioned with 37% phosphoric acid for 15 s and a composite resin restoration (TPH Spectrum) was fixed using a one-bottle adhesive system (Adper Single Bond) and a dual-cured resinous cement (Rely X ARC) under a load of 500 g for 5 minutes. The samples were serially cut perpendicular to the bonded interface to obtain slices of 1.2 mm in thickness. Each slab was trimmed with a cylindrical diamond bur resulting in an hourglass shape with a cross-sectional area of approximately 1 mm². The microtensile bond strength (µTBS) testing was performed in a testing machine (EMIC 2000 DL) at a 0.5 mm/minute crosshead-speed until failure. After fracture, the specimens were examined under SEM to analyze the mode of fracture. µTBS Means were expressed in MPa and the data were analyzed by two-way ANOVA (3X3) and the Tukey test (α = 0.05). The storage times of 7 and 30 days produced no significant difference irrespective of the solution type. The formalin and thymol solutions, however, did have a negative influence on bond strength when the teeth were stored for 6 months.

Descriptors: Composite resins; Tensile strength; Thymol; Formaldehyde; Sodium azide; Dentin.
Introduction

The mechanism of bonding to acid-etched dentine is described as micromechanical, generated by the infiltration of monomers through the dematerialized dentine surface, enveloping the exposed collagen network, where it polymerizes in situ, creating a mixed structure named the hybrid layer. The clinical success of indirect restorative procedures is directly related to the performance of the process of fixation and related to the material and standard of formation of the hybrid layer.

Several mechanical tests as shear, micro-shear, tensile and microtensile tests have been used to characterize the bond strength of adhesive systems to dentin. Since its introduction in 1994, the microtensile technique has proven to be a very useful tool for measuring the bond strength of adhesive materials to dental tissues. Major advantages of the microtensile technique include improved stress distribution during adhesion testing and the ability to perform the test in very small specimens. Bonding tests, mainly those involving indirect restorations, require a long time between sample preparation and the end of the test. This extended time can result in dentine structural changes after extraction of the tooth, with proven consequences in the test’s results.

The teeth used for in vitro bonding studies are mainly obtained from humans and bovines. Teeth from these two sources are contaminated with bacteria. Thus, the potential for the transmission of communicable diseases via blood-borne pathogens, particularly from human teeth, is a concern. It is therefore important that these teeth be decontaminated in a sterilizing medium before any bond-strength tests are done in the laboratory. A variety of media that possess bactericidal and bacteriostatic properties have been used for storage purposes. Some of the solutions most commonly used as storage media for in vitro studies are formalin, thymol and sodium azide. The media in which teeth are stored after harvesting and the duration of storage, however, may influence the bond-strength results.

The present study was performed to evaluate the influence of the method and period of teeth storage on the adhesive bond strength of indirect composite resin restorations to bovine dentine by microtensile bond testing. The null hypotheses were that storage-time and storage medium do not influence the bond strength of the adhesive system to dentin.

Material and Methods

Ninety bovine recently extracted incisors were selected, cleaned by removing calculus and soft-tissue deposits with a hand scaler, and then by applying a rubber cup and fine pumice water slurry. The teeth were stored in the 3 solutions most commonly used as storage media: 0.2% thymol, 10.0% formalin and 0.2% sodium azide, during 3 periods of storage: 7 days, 30 days and 6 months, resulting in nine experimental groups (n = 10). After the storage time, the teeth were removed from their respective media and were stored in distilled water at 4°C until completion of the experiment. The roots were cut 2 mm bellow the cementum-enamel junction using a diamond disk (KG Sorensen, Barueri, SP, Brazil), and the buccal surface was ground with #600-grit silicon carbide paper (Noritake, Campinas, SP, Brazil) to obtain a flat dentin surface with a standardized smear layer. Composite resin restorations with 4 mm in length, 3 mm in width and 5 mm in thickness were constructed using a hybrid resin composite (TPH Spectrum, Dentsply Caulk, Milford, DE, USA). The restorations were sandblasted with 50 µm aluminium oxide airborne-particle abrasion at 1-bar pressure for 10 s (Bioart, São Carlos, SP, Brazil) and treated with a silane coupling agent for 1 minute (Ceramic Primer, 3M-Espe, St. Paul, MN, USA). The dentin was etched using 37% phosphoric acid (3M-Espe, St. Paul, MN, USA) for 15 seconds, rinsed, and blotted dry with absorbent paper. With a fully saturated brush tip, 2 consecutive coats of an adhesive system (Adper Single Bond, 3M-Espe, St. Paul, MN, USA) were applied to the tooth and polymerized with a halogen light-polymerization unit (XL 3000; 3M-Espe, St. Paul, MN, USA) for 20 seconds at an intensity of 800 mW/cm². A dual-cured resinous cement (Rely X ARC, 3M-Espe, St. Paul, MN, USA) was then dispensed onto a mixing pad and mixed for 10 seconds. A thin layer of the material was applied to the resin restoration,
which was seated in place. Resinous cement excess was removed with a brush and was polymerized from each face for 40 seconds.

The samples were stored for 24 hours in distilled water at 37°C. After that, the samples were serially sectioned perpendicular to the bonded interface using a sectioning machine (Isomet 1000, Buehler Ltd., Lake Bluff, II., USA) to obtain several slices of approximately 1.2 mm in thickness. Each slab was trimmed to an hourglass-shape with a superfine diamond bur under air-water irrigation with a cross-sectional area of approximately 1 mm². An average of four hourglass shaped specimens from the same tooth was obtained for each group. Many hourglass shaped specimens were unable to be tested due to debonding before placement on the testing machine (sodium azide: 7 days – 4 specimens debonded before microtensile testing; 30 days – 4; 6 months – 4; thymol: 7 days – 3 specimens; 30 days – 2; 6 months – 10; formalin: 7 days – 4 specimens; 30 days – 4; 6 months – 11). The specimen was fixed to the grips of the microtensile testing device with cyanoacrylate glue (Loctite Super Bonder, Henkel Loctite Corporation, Rocky Hill, CT, USA). Then they were submitted to a microtensile test at a 0.5 mm/min speed in a testing machine (EMIC 2000 DL, São José dos Pinhais, Paraná, Brazil) until failure. After fracture, the specimen was removed from the testing apparatus and the cross-sectioned area at the site of fracture was measured with a digital caliper (S235, Sylvac SA, Crissier, Vaud, Switzerland). The data were expressed in MPa. Statistical analysis aimed to determine the influence of 2 factors involved in this study, the storage method and the time elapsed between tooth extraction and accomplishment of the bond test. Therefore, the data were analyzed with two-way ANOVA and the Tukey HSD test ($\alpha = 0.05$). The statistical analysis was made considering each tooth as being a sample in the accomplishment of the microtensile test. From this, an average of the results of the hourglass shaped specimens obtained for each tooth was extracted, considering the existence of a correlation between slices from the same tooth. Analysis was performed excluding the hourglass-shaped specimens that debonded before the microtensile testing.

The fractured samples were dematerialized in an 18% chloridric acid solution for 5 s and ultrasonically cleaned in deionized water for 10 min. After that, they were immersed in a 1% sodium hypochlorite solution for 10 min for deproteinisation, and ultrasonically cleaned in deionized water for 10 min. The samples were fixed in 2.5% glutaraldehyde for 12 hours at 4°C and then dehydrated in alcohol 50% for 10 min, 70% for 10 min, 95% for 10 min and 30% for 30 min. They were allowed to air-dry for 12 h, sputter-coated with gold (MED 010, Balzers, Balzer, Liechtenstein) and examined under SEM (LEO 435 VP, Leo Electron Microscopy, Cambridge, Cambridgeshire, UK).

Results

Microtensile bond strength (μTBS) means and standard deviations for all groups are shown in Table 1. Two-way ANOVA showed that there were significant differences for the interaction between both factors. Teeth storage into sodium azide medium produced similar bond strength values irrespective of time storage. Thymol for seven days or thirty days and formalin for seven days or thirty days also produced similar values. Nonetheless, teeth stored in thymol or formalin for six months had μTBS values significantly lower than those of the other time periods. The groups with lower μTBS values also had higher proportions of spontaneously debonded hourglass shaped specimens.

Failure mode observations made by SEM showed variation among groups. Cohesive failure in resinous cement and/or in composite and interfacial and/or top of the hybrid layer failure could be observed in all the groups (Figures 1-3).

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<th>Table 1 - μTBS Means (MPa) and distribution by statistical categories.</th>
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(Different letters represent significant differences ($p < 0.05$) identified by the Tukey test. The capital letter represents analysis within the column, and the lower case letter, within the row).
Discussion

The null hypotheses were rejected. The results demonstrated a negative influence on μTBS values of the one bottle adhesive system to bovine dentin when the thymol and formalin media were used for 180 days. The existing reports on the effects of storage medium and duration of storage on the shear-bond strength of resin to dentine are equivocal. Furthermore, there is little fundamental information available on the structural alterations of dentine in various solutions. Several studies reported that storage medium or time of storage had no significant effect on the shear-bond strength of resin to dentine.\textsuperscript{11,13,14} These studies appear to support the results of the present study when the periods of 7 days and 1 month were considered, since the storage of the bovine teeth had no influence on bond strength irrespective of the storage solution up to the period of one month. However, when the teeth were stored for six months there was a significant difference among the solutions. Teeth stored in formalin or thymol for six months had microtensile bond strengths significantly lower than those of the other groups. In 1991, two review articles were published which stated that for dentin adhesive studies, the teeth must be kept in a moist environment to prevent dehydration and should not be stored longer than 6 months.\textsuperscript{15,16}

Several studies related that formalin had no significant time effect on dentin bond strengths.\textsuperscript{8,11,17} On the other hand, other studies reported that teeth stored in formalin had higher dentin bond strengths.\textsuperscript{18,19} The increase of dentin bond strength and microleakage decrease for teeth stored in formalin are a result of collagen cross-linking by formaldehyde. Formaldehyde, being a monofunctional aldehyde, exhibits good penetration into tissues and less cross-linking of proteins than glutaraldehyde.\textsuperscript{20} However, Jameson \textit{et al.}\textsuperscript{21} (1994) suggested that the mechanical behavior of the covalently cross-linked type I collagen in dentine is not significantly affect-
ed by storage of teeth in neutral-buffered formalin. Supporting the results of the present study, Wieczkowski et al. (1989) observed bond strength values significantly lower for teeth stored for a year in formalin in relation to teeth stored for 24 h, 48 h, and 1 week. Formalin is effective as a high-level disinfectant if the teeth are in the solution for at least 2 weeks, but it cannot be recommended as a storage medium for dentin bonding studies due to the variability in dentin bond strengths. Formalin storage may alter the structure of the dentin and affect the way in which dentin bonding agents adhere to it. Thymol produced similar microleakage results when compared with freshly extracted teeth. It was not recommended as a storage solution by the investigators, however, since it is a phenolic compound suspected of inhibiting polymerization of methacrylates. Water with thymol and phosphate-buffered saline with thymol would have no effect on either the organic or inorganic content of dentine. Neither solution acts as a fixative.

Changes occurred in the surface of dentine as a function of storage solution and time as measured by FTIR (Fourier transform spectroscopy). The changes seen were greatest in the mineral component, possibly due to changes in the pH of the solutions and/or deposition of components of the solutions onto the dentine surface. There were minimal changes detected in the organic portion of dentine. The optical properties of dentine did not significantly change, compared to the baseline, regardless of the storage solution and time at any wavelength. Jameson et al. (1994) found an increase in weight loss after 12 weeks of storage in neutral-buffered formalin. They suggest that there may have been some dissolution of the smear layer or surface dentine mineral. The related changes in the mineral component of dentine are a possibility that would lead to the decreased bonding observed in the present study when the teeth were stored for six months. Even though these changes are minimal, the storage for six months would have more influence on these changes than the storage for a week or a month. It is also apparent that further investigations are needed to examine what postmortem changes occur in dentine, whether these changes are modified by various storage conditions, and whether they have any significant effect on bonding of resin composites. It can thus be observed that the action of the solutions used in tooth storage, considering the period of storage, is not yet clearly defined, rendering an analysis of the effect of these solutions on bond strength values difficult. However, aiming to obtain more realistic results in in vitro tests, short storage times should be used, thus minimizing the negative influence on the dentinal structure.

Conclusions

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

- The storage times of 7 and 30 days produced no significant difference in µTBS, irrespective of solution type, although the formalin and thymol solutions had a negative influence on bond strength when the teeth were stored for 6 months.
- It is recommended that research using microtensile bond strength to dentine testing should use tooth storage for no longer than 1 month.

Acknowledgments

The authors are indebted to Dr. E. W. Kitajima (NAP-MEPA/ESALQ-USP) for the SEM technical support. This study was supported by grants from FAPEMIG (grant n. D.008/2004.)

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