Effect of storage and disinfection methods of extracted bovine teeth on bond strength to dentin

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Received for publication: May 14, 2007
Accepted: August 07, 2007

Abstract
The aim of this study was to evaluate the effect of storage and disinfection methods (SDM) on bond strength (BS) to bovine dentin, using two adhesive systems: Adper Single Bond and Clearfil Protect Bond. Method: Extracted bovine teeth were assigned to the following SDM: 100% Humidity (HU); Gamma Radiation (GR); Autoclave (AU); 0.10g/mL Thymol (TH); 10% Formalin (FO); Frozen (FR); 0.2% Sodium Azide (SA) and 0.5% Chloramine T (CT) (n=10). The GR and AU groups were submitted to sterilization methods and stored at 100% humidity for 24 hours at 37°C, before testing. TH, FO, FR, SA and CT groups were stored for three months at 5°C, except for FR (-4°C). The adhesive systems were applied according to manufacturer’s instructions. For BS testing, the micro-shear test was performed, using Tygon mold (0.75mm diameter and 1mm high), which was filled with Clearfil AP-X (Kuraray) composite resin. Data were analyzed by two-way ANOVA and Tukey Test (p<0.05). BS of adhesive systems was similar for all SDM, except for 10% formalin. HU, GR, AU, TH and SA did not change the BS for the adhesives tested. For most of SDM, BS of adhesive systems was similar. Some 3 month-SDM groups (FR, FO and CT) reduced the BS.

Key Words:
dentin, disinfection, sterilization, dentin-bonding agents

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Introduction

The difficulty with in vivo bond strength test or any research with bonding agents has led to use of extracted teeth for in vitro testing. Because extracted teeth are a potential source for cross-contamination to laboratory equipments and personnel, the teeth must be decontaminated and stored. Some solutions for storage and disinfection or sterilization methods to treat extracted teeth have been indicated. They can be observed in publications of dental literature, which evaluate the effectiveness of sterilizing methods and the behavior of bonding agents applied after storage and disinfections.

Although these storage and disinfection methods render ideal conditions for extracted teeth handling, there are some concerns about compositional or structural alterations of enamel and dentin. As the success of bonding procedures depend on integrity of enamel and dentin components after the treatments, the storage and disinfection methods must conserve the teeth as were freshly or immediately after extraction. Conversely, any change promoted by treatments can compromise the performance of adhesive systems, affecting the results of the study.

Present methods of storing and sterilizing extracted teeth include steam autoclave, freezing, gamma radiation, liquid chemicals (water, thymol, formalin, sodium azide, chloramine T, hypochlorite, and glutaraldehyde) and gaseous chemical (ethylene oxide). Considering all variability of tooth preparation, storage conditions, this study aimed to evaluate the effects of storage and disinfection methods to treat freshly extracted teeth on bond strength to bovine dentin.

Material and Methods

Tooth Preparation

Eighty freshly extracted bovine incisors were used in this study. After extraction, all teeth were immediately cleaned, pumiced and randomly assigned to eight experimental conditions as described in Table 1. Each tooth was stored or kept separately in a dark container (25 mL), until sterilized by autoclave or freezing. Teeth kept in 100% humidity measured by hygrometer (Thermo-Hygrometer, TFA Dostmann, Wertheim-Reichoizheim, Germany) until sterilized by autoclave or gamma radiation were tested after 24 hours and teeth stored with formalin, sodium azide, chloramine T, hypochlorite, and glutaraldehyde and gaseous chemical were tested after 24 hours and teeth stored with liquid chemicals were tested after 24 hours and teeth stored with gaseous chemical were tested after 3 months were tested after the storage period.

Buccal and lingual enamel surfaces were wet-abraded with 200-grit SiC paper (Carborundum, Vinhedo, SP, Brazil) to remove the enamel and create parallel dentin flat surfaces. The roots were removed using a diamond disk (Isomet, Buehler, Evanstone, IL, USA). Each dentin slice of the crown was sectioned longitudinally and divided into two parts with similar size (12 mm in length X 5 mm in width X 1.0 mm in thickness). Each part was used with an adhesive system. The buccal flat dentin surfaces were wet-abraded with 600-grit SiC paper (Carborundum) to create a standardized smear layer and the dentin fragments were randomly divided into 16 groups (two adhesive systems and eight methods of storage and disinfection).

Shear Bond Strength Testing

Two adhesive systems were selected: a two-step self-priming system (Clearfil Protect Bond, Kuraray Medical Inc., Kurashiki, Okayama, Japan) and a two-step etch & rinse single bottle adhesive (Adper Single Bond, 3M ESPE, St. Paul, MN, USA). The methodology developed by Shimada et al. and modified by Giannini et al. was used to prepare specimens for the micro-shear test.

Adhesive systems were applied to dentin, according to the manufacturer’s instructions. Three cylindrical translucent molds (Tygon tubing, TYG-030, Saint-Gobain Performance Plastic, Maine Lakes, FL, USA), were positioned over the bonded dentin of each dental fragment and Clearfil AP-X composite resin (Kuraray Medical Inc.) was applied into the molds to fill their internal volume, using a composite instrument (#1/2, Duflex - SS White, Rio de Janeiro, RJ, Brazil). The molds filled with composite were light-irradiated with a curing unit (XL 3000, 3M ESPE) for 40 seconds. Afterwards, the tube molds were removed to expose the resin composite cylinders (0.7 mm diameter by 1.0 mm high/0.38 mm²) bonded to the dentin surfaces, thus, three bonded small resin cylinders were obtained for each dental fragment.

Composite cylinders were checked under an optical microscope (30X) (EMZ-TR, Meiji Techno Co., Saitama, Japan). The cylinders that showed no apparent interfacial defects or bubble inclusion and no leaking of composite were tested. The restored dental fragments were stored in distilled water at 37 °C for 24 hours. Each dental fragment was attached to the testing device with cyanoacrylate glue (Super Bonder, Loctite, Itapevi, SP, Brazil) and each composite cylinder tested in a universal testing machine (4411, Instron Corp., Canton, MA, USA). A shear load was applied to the base of the composite cylinder with a thin wire (0.2 mm diameter) at a crosshead speed of 0.5 mm/min until failure. The micro-shear bond strengths were calculated and expressed in MPa. Three bond strength measurements per dental fragment were recorded and the bond strength (BS) mean was determined for each fragment. The mean values for each experimental group were calculated from the bond strength mean of each dental fragments (n = 10), which were obtained from three composite cylinders bonded to dentin. The results were statistically analyzed by two-way (adhesive system X storage / disinfection method) analysis of variance (ANOVA) and Tukey test at the 5% level of significance. The statistical analysis was done using SAS 9.0 (SAS Institute, Cary, NC, USA).
Results

Shear bond strength means are presented in Table 2. Two-way ANOVA showed that there were statistically significant differences for the factor “adhesive system” and for the factor “storage / disinfection method”. In addition, it identified a significant interaction between the two factors. Tukey test showed significant differences between experimental groups (p<0.05).

Comparing the bond strength between adhesives, only the treatment with formalin 10% significantly decreased the bond strength of Clearfil Protect Bond to dentin. Regarding storage and disinfection conditions for both adhesive systems, none of the experimental conditions presented significant difference when compared to the teeth that were kept in 100% humidity after extraction. For Single Bond, only 10% formalin had higher values than teeth that were frozen or stored in 0.5% chloramine T. For Clearfil Protect Bond, teeth sterilized in autoclave showed higher values of BS than those frozen or stored in 10% formalin. Teeth stored in 2% sodium azide also presented significant difference when compared to 10% formalin.

Discussion

Studies of the dentin structure and investigations of approaches to dentin bonding require the use of extracted teeth, which must be unaltered at the time of evaluation. As the teeth need to be sterilized or disinfected and stored until testing, they may undergo changes in structure and composition during the treatments8,10,14,37, which can interfere with results. The adhesive systems used in this current study comprised two different approaches to bonding to tooth hard tissues: a two-step self-etching primers (Clearfil SE Bond) and an etch-and-rinse system (Single Bond)13,21. However, they showed similar bond strengths when the bovine teeth were submitted to the eight proposed storage and disinfection conditions after extraction, except for the storage in 10% formalin. Teeth kept in 100% humidity served as control group, because they were freshly and not submitted to long storage time and no sterilization or disinfection method. All proposed sterilization and disinfection methods in this study showed similar bond strength to control group (100% humidity) for

Table 1 - Storage and disinfection conditions.

<table>
<thead>
<tr>
<th>Conditions (Manufacturers)</th>
<th>Protocol for each condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% Humidity (Thermo-Hygrometer, TFA Dostmann, Wertheim-Reicholzheim, Germany)</td>
<td>Teeth were kept in 100% humidity for 24 h at 37°C.</td>
</tr>
<tr>
<td>Gama Radiation (Gammacell 220, MDS Nordion, Ottawa, ON, Canada)</td>
<td>Teeth were sterilized by gama radiation for 24h under 14.5 KGrey.</td>
</tr>
<tr>
<td>Autoclaving (2340MK, Tuttnauer, Haupppage, NY, USA)</td>
<td>Teeth were sterilized in autoclave for 20 min at 121°C and stored in 100% humidity for 24 h at 37°C.</td>
</tr>
<tr>
<td>10% Formalin (Proderma, Piracicaba, SP, Brazil)</td>
<td>Teeth were stored for 3 months in 10% formalin solution at 5°C.</td>
</tr>
<tr>
<td>0.10g/mL Thymol (Synth, Diadema, SP, Brazil)</td>
<td>Teeth were stored for 3 months in 0.10g/mL thymol solution at 5°C.</td>
</tr>
<tr>
<td>Freezing (Biplex, Consul, Joinvile, SC, Brazil)</td>
<td>Teeth were frozen at -4°C for 3 months.</td>
</tr>
<tr>
<td>0.5% Chloramine T (Merck, Darmstadt, Germany)</td>
<td>Teeth were stored for 3 months in 0.5% chloramine T solution at 5°C.</td>
</tr>
<tr>
<td>2% Sodium Azide (Merck, Darmstadt, Germany)</td>
<td>Teeth were stored for 3 months in 2% sodium azide solution at 5°C.</td>
</tr>
</tbody>
</table>

Table 2 - Bond strengths means (SD), in MPa, for adhesive systems as a function of storage and disinfection conditions.

<table>
<thead>
<tr>
<th>Storage and disinfection conditions</th>
<th>Adhesive System</th>
<th>Clearfil Protect Bond</th>
<th>Adper Single Bond</th>
</tr>
</thead>
<tbody>
<tr>
<td>Humidity</td>
<td></td>
<td>28.8 (9.7) Aabc</td>
<td>32.4 (11.2) Aab</td>
</tr>
<tr>
<td>Autoclaving</td>
<td></td>
<td>37.7 (10.7)Aa</td>
<td>32.3 (9.0) Aab</td>
</tr>
<tr>
<td>Gama Radiation</td>
<td></td>
<td>29.6 (5.7) Aabc</td>
<td>33.4 (13.5) Aab</td>
</tr>
<tr>
<td>Thymol</td>
<td></td>
<td>27.4 (10.9)Aabc</td>
<td>26.5 (7.4) Aab</td>
</tr>
<tr>
<td>10% Formalin</td>
<td></td>
<td>17.4 (5.3) Bc</td>
<td>38.2 (11.1) Aa</td>
</tr>
<tr>
<td>Freezing</td>
<td></td>
<td>18.8 (8.9) Abc</td>
<td>22.8 (7.6) Ab</td>
</tr>
<tr>
<td>2% Sodium Azide</td>
<td></td>
<td>30.0 (7.9) Aa</td>
<td>34.0 (9.1) Aab</td>
</tr>
<tr>
<td>0.5% Chloramine T</td>
<td></td>
<td>28.5 (7.9) Aabc</td>
<td>22.3 (8.2) Ab</td>
</tr>
</tbody>
</table>

Groups having different letters (upper case=row; lower case=column) are significantly different (p<0.05).
Gamma radiation is currently used in laboratory research to sterilize extracted teeth and it is used in research and preclinical dental laboratory. Pashley et al. reported that the dentin permeability and bond strength were not affected by autoclaving procedure. However, spectroscopy measurements have shown alterations on tooth structure and on mineral and organic contents. The autoclaving process provided high bond strength values and for Single Bond it was similar to other treatments. For the self-etching process provided high bond strength values and for Single Bond adhesive is included among etch & rinse systems and showed higher bond strength to dentin, which was not observed for etch and rinse system. The etch & rinse adhesive system is used after phosphoric acid etching and this conditioning can remove the altered superficial layer, which could influence the bonding. As self-etching systems are applied directly to dentin, the altered superficial dentin can affect the bond strength because interfere with bonding mechanism.

Chloramine T solution has been recommended as infection controlling for research purposes, because it did not show adverse effect on organic phase of dentin. Also, the microleakage tested around composite restoration was similar to freshly extracted teeth. However, the results of this study and the investigation of Tittley et al. showed that the chloramine T solution was not the best condition for storage of extracted teeth.

Similarly, conflicting results are observed after storage in thymol solution. Since thymol is a phenolic compound, it have not recommended as a storage solution because can inhibit the polymerization of methacrylates. Moreover, changes on superficial composition and on bond strength to dentin have been observed. However, no effect on dentin permeability, microleakage and bond strength was related, which considered thymol solution suitable for storage of extracted teeth.

Sodium azide is a salt of hydrazoic acid and its solution is used to prevent bacterial growth. This disinfection method did not alter the bond strength to dentin and no effect of sodium azide storage on structure or composition of enamel and dentin has been described in dental literature. According to Tittley et al., storage of extracted teeth by freezing immediately after extraction is an effective method to conserve them. It is possible that post mortem changes could occur in dentin, which in turn can affect the outcome of tests for bond strength. Freezing is preferred methods of storage for some studies, however, it did not show good results in this current study.

ISO report recommends that the teeth must be cleaned and pumiced immediately extraction and stored in distilled water or in 0.5% chloramine solution, for at least 1 week. However, a variety of media that possess bactericidal and bacteriostatic properties have been indicated for storage purposes, which impair the standardization of bond strength tests. Although the results of this study did show significantly differences in bond strength between teeth more freshly (100% humidity group) and stored or disinfected teeth, all reports of alterations of teeth must be considered before choosing the storage and disinfection method. The bond strength test is not the specific methodology to analyze the compositional and the structural changes after storage, however, if some more significant changes occurred, they could interfere with bond strength values.

Acknowledgements
This study was supported by grants from PIBIC/CNPq and by grants from 301769/2004-4 from the CNPq, Brazil.

References