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Effect of Refrigeration on Bond Strength of Self-Etching Adhesive Systems

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The purpose of this study was to investigate the tensile bond strength to dentin of three self-etching adhesive systems at refrigerated and room temperatures. Seventy-eight bovine incisors were embedded in self-cured acrylic resin, abraded on a water-cooled lathe and polished with 400- and 600-grit sandpapers to obtain standard dentin surfaces. The specimens were randomly assigned to 6 groups (n=13). Clearfil SE Bond, AdheSE and One-Up Bond F adhesive systems at refrigerated (4ºC) and room temperatures (23ºC) were applied to dentin according to the manufacturers' instructions. A truncated composite resin (Herculite XRV) cone was bonded to dentin surface. The specimens were stored in distilled water at 37ºC for 24 h and submitted to tensile bond strength testing at a crosshead speed of 0.5 mm/min. Means in MPa were analyzed statistically by Student's t-test at 5% significance level. No statistically significant differences ($p>0.05$) were found between the adhesive systems applied at refrigerated and room temperatures. In conclusion, no adverse effects on tensile bond strength were observed when self-etching adhesive systems were used after being taken directly from the refrigerated storage.

Key Words: adhesive systems, bond strength, refrigeration, room temperature.

INTRODUCTION

Several generations of adhesive materials had been developed until the advent of hydrophilic systems. Dentin bond strength of some of the currently available adhesive systems approaches or even exceeds enamel bond strength (1,2).

New adhesive systems with different chemical compositions have been continuously introduced. Together with the search for more efficient systems, the manufactures have also attempted to reduce the number of material components. Conventional (multiple-bottle) adhesive systems include a conditioner, such as phosphoric acid, a primer and an adhesive, which are all applied in separate steps. To simplify their application technique, some manufactures have combined the primer and adhesive in one bottle. New approaches for either enamel or dentin bonding without phosphoric acid pretreatment have been proposed with the introduction of self-etching adhesive systems. The two-step self-etching adhesive systems are characterized by separate chemical formulations for priming and bonding by using a self-etching hydrophilic primer. Therefore, etching and priming are performed simultaneously. The primed surfaces are subsequently coated with a light-cured hydrophobic adhesive layer. The all-in-one adhesives,

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or one-step self-etching adhesive systems, are the ultimate advancement in adhesive technology: they simultaneously etch, prime and bond following a single application. These systems are extremely hydrophilic as they contain high concentrations of both ionic and hydrophilic monomers (3).

Regardless of the adhesive system, one of the factors influencing the clinical performance of a restoration is how it is placed by the dentist.

Although most manufacturers recommend that the adhesive materials should be stored at room temperature, these materials are usually refrigerated in order to extend their shelf life. In practice, most dentists take the materials from the refrigerator and use them immediately, without allowing time for the material to reach room temperature. Reduced temperatures influence the properties regarding the curing efficiency, such as microhardness and diametral tensile strength, due to the decreased in polymerization (4). Upon refrigeration, the viscosity of the polymeric material increases and the penetration of the adhesive into the dentin surface may be reduced as well as its bond strength. In addition, refrigeration may influence the evaporation of primer solvents, which also reduces the bond strength. It has also been demonstrated that incomplete solvent evaporation affects adversely the sealing ability of adhesive systems (5). Concerning the total-etch adhesive systems, Spohr et al. (6) demonstrated that the temperature had no influence on bond strength. However, it is important to investigate whether the temperature can influence the bond strength of self-etching adhesive systems.

The purpose of this study was to assess the tensile bond strength to dentin of two 2-step self-etching adhesive systems and one all-in-one adhesive system at refrigerated and room temperatures. The null hypothesis is that refrigeration does not influence the bond strength of the adhesive systems to dentin.

MATERIAL AND METHODS

Seventy-eight bovine incisors were cleaned of gross debris and stored in distilled water at 4ºC until use within 6 months. The roots were removed and some portions of the crown were cut-off from each tooth using a water-cooled double-faced diamond disc (KG Sorensen, São Paulo, SP, Brazil) to obtain a 5x5-mm fragment from the central area of the buccal surface. Each tooth section was fixed onto a glass plaque with wax. A metallic cylindrical device was placed on the glass slab over the fragment, which was centralized, and the device was filled with a self-cured acrylic resin (Jet Classico, São Paulo, SP, Brazil). Two millimeters of acrylic resin and tooth were removed together to expose a flat dentin surface using a water-cooled lathe (Nardini-ND 250 BE, São Paulo, SP, Brazil). Dentin was ground and polished using 400- and 600-grit sandpapers (Carborundum Abrasivos, Recife, PE, Brazil) on an automated polisher APL-4 (Arotec Ind. Com Ltda, São Paulo, SP, Brazil) to obtain standard dentin surfaces.

After polishing, the embedded teeth were randomly assigned to 6 groups (n=13) for bonding with three self-etching adhesive systems at refrigerated (4^{o}C) and room temperature (23^{o}C). Prior to surface treatment, the bonding area was demarcated by attaching a piece of vinyl tape measuring 3 mm in diameter (3M Dental Products Division, St. Paul, MN, USA) to specimen surface. Clearfil SE Bond (Kuraray Corp., Tokyo, Japan), AdheSE (Ivoclar Vivadent, Shaan, Liechtenstein) and One-Up Bond F (Tokuyama Corp., Tokyo, Japan) adhesive systems were applied according to the manufacturers' instructions.

Clearfil SE Bond: the primer was applied to the dentin surface for 20 s and excess was removed with a cotton bud, leaving a moist surface. Two consecutive coats of adhesive were applied using a saturated disposable brush tip. After gentle air drying for 5 s, the material was light-cured for 10 s using a visible light-curing unit at 600 mW/cm^2 (XL 1500; 3M Dental Products Division); *AdheSE:* the primer was applied to dentin for 15 s and excess was removed with a cotton bud, leaving a moist surface. Two consecutive coats of adhesive were applied using a disposable saturated brush tip. Solvent was evaporated with a mild air stream for 5 s, and the adhesive system was light-cured for 10 s. O*ne-Up Bond F*: parts A and B were mixed and applied to dentin for 20 s. Excess was removed with a cotton bud. Next, the material was gently air-thinned for 5 s and light-cured for 10 s.

A 4-mm high metallic cylindrical device with 3-mm-diameter opening at the base and 5-mm-diameter opening at the top was placed onto the specimen so that smaller diameter was coincident with the treated dentin area. A composite resin (Herculite XRV; Kerr Corp., Orange, CA, USA; shade A2), was inserted into the matrix cavity in two increments, each of them light-cured for 40 s. An inverted composite resin cone was obtained to provide a grip for the hook used in the tensile bond test machine.

The specimens were stored in distilled water at 37ºC for 24 h and submitted to tensile bond strength testing using a universal testing machine (DL-3000; EMIC, São José dos Pinhais, PR, Brazil) at a crosshead speed of 0.5 mm/min until failure occurred. Tensile bond strength means (in MPa) were calculated by dividing the failure peak load by the specimen surface area. Student's t-test was used to compare the tensile bond strength means for each adhesive system at 4º C and 23º C at 5% significance level.

After testing, the surfaces of the fractured specimens were examined with a stereomicroscope (Olympus Corp., Tokyo, Japan) at X20 magnification. The types of failure occurred during debonding were classified as follows: *adhesive*, if the composite resin cone had fractured at the adhesive-tooth interface; *cohesive in dentin*, if the composite resin cone had fractured with a large portion of dentin attached; *cohesive in resin,* if the composite resin cone had fractured inside the composite resin; or *mixed*, a combination of adhesive and cohesive in dentin or cohesive in resin.

RESULTS

Tensile bond strength means for the tested self-ecthing adhesive systems are shown in Table 1.

No statistically significant differences were found between the room and refrigerated temperature groups ($p>0.05$). A trend towards higher bond strengths was noted with refrigerated Clearfil SE Bond and One-Up Bond F, but this difference from the other groups was not statistically significant.

The examination of the debonded specimens under stereomicroscopy showed a predominance of adhesive failures for all adhesive systems at both room and refrigerated temperatures (Table 2). Regarding the mixed failures, the combination of adhesive failure and cohesive failure in dentin was more commonly observed than the combination of adhesive failure and cohesive failure in resin. No cohesive failure in dentin alone or cohesive failure in resin alone was observed.

Table 1. Tensile bond strength means (MPa) to dentin of the three adhesive systems at room and refrigerated temperatures.

Adhesive System	n	Room Temperature (23ºC)	Refrigerated Temperature (4º C)	t values	*p*
Clearfil SE Bond	13	2.44 ± 1.45 a	3.37 ± 1.98 a	-1.35	0.19
AdheSE	13	9.49 ± 2.75 a	9.51 ± 2.34 a	-0.02	0.98
One-Up Bond F	13	3.22 ± 2.59 a	4.62 ± 2.62 a	-1.37	0.18

Means followed by same letter in rows indicate no statistically significant difference at 95% confidence level (Student's t-test, $p<0.05$).

Table 2. Failure mode analysis of the debonded specimens.

	Adhesive	Mixed (adhesive/ cohesive in resin)	Mixed (adhesive/ cohesive in dentin)
AdheSE			
room temperature	10	0	3
refrigerated temperature	9	0	4
One-Up Bond F			
room temperature	11	0	2
refrigerated temperature	10	0	2
Clearfil SE Bond			
room temperature	9	1	3
refrigerated temperature	11	0	2

DISCUSSION

After the introduction of self-etching adhesive systems, several studies have discussed degradation and water movement across bonded dentin (7,8). However, the influence of temperature on bond strength has not yet been evaluated. Clearfil SE Bond and AdheSE are adhesive systems that combine etching and priming in one step, before the placement of a layer of a hydrophobic light-cured adhesive system. One-Up Bond F is an all-in-one self-etching adhesive system that etches, primes and bonds the substrate simultaneously with a single application. In this study, the temperature of the adhesive systems had no significant influence on the tensile bond strength to dentin. Therefore, the null hypothesis was confirmed.

Several studies have reported the influence of both temperature and relative humidity on early bond strength to dentin (9,10). Hagge et al. (11) investigated the shear bond strength of adhesive systems kept at refrigerated (4ºC) and room (23ºC) temperatures. Although no difference was found between Prime & Bond and All-Bond 2, the shear bond strength of Scotchbond Multi-Purpose was higher at refrigerated temperature (13.14 MPa) than at room temperature (5.52 MPa). However, no satisfactory explanation for the greater bonding ability of Scotchbond Multi-Purpose maintained at 4ºC was given by the authors. Spohr et al. (6) studied the effect of refrigeration on a conventional adhesive system (Scotchbond Multi-Purpose) and two simplified adhesive systems (Single Bond and Prime & Bond NT). No adverse effect on bond strength was observed when these materials were applied to dentin at either 4ºC or 23ºC.

The type of solvent used in the primer is important for bond strength (12). Clearfil SE Bond and AdheSE have water-based primers, whereas One-Up Bond F has an acetone-based primer. The evaporation of these solvents after application to dentin is extremely important (5). The results of this study indicate that refrigeration probably did not affect solvent evaporation because no difference was observed in the tensile bond strengths of the tested adhesive systems. Additionally, another theoretical, disadvantage of refrigeration, i.e., the decreased bond strength due to the adverse effect of refrigeration upon the physical properties of the adhesive materials, seemed not to have occurred because little or no effect could be detected in bond strength under refrigerated conditions. The time necessary to apply the adhesive material to the substrate is probably enough to attenuate the low temperature and reduce the adverse effect of refrigeration. In addition, the oral temperature (37ºC) is usually higher than the room temperature, which is a positive factor.

Refrigeration had not an adverse effect on the mode of failure. Spohr et al. (6) and Hagge et al. (11) reported a predominance of mixed-failure mode with refrigerated adhesive systems after testing shear bond strength. The different failure modes reported in previous studies may be explained by the methodologies used, i.e., tensile bond strength *versus* shear bond strength. Stresses at tooth adhesive/ interface are far from being homogeneous and are highly dependent on test geometry and loading configuration employed (13).

In this study, the adhesive systems were applied to bovine dentin. The use of bovine teeth is justified by the difficulty in obtaining human teeth mainly because of the bioethical issues in research involving humans. In addition, previous studies have shown similar bond strength for human and bovine dentin (14,15).

Within the limitations of this investigation, it may be concluded that no adverse effects occurred when self-etching adhesive systems were used after being taken directly from refrigerated storage. However, extrapolations from the findings of this study cannot be made to other adhesive systems. Further research with different materials should be conducted.

RESUMO

O objetivo deste trabalho foi avaliar a resistênciade união à dentina de três sistemas adesivos autocondicionantes quando estes se encontravam refrigerados ou à temperatura ambiente. Setenta e oito incisivos bovinos foram embutidos em resina acrílica autopolimerizável, desgastados em um torno mecânico sob refrigeração à água, polidos com papel abrasivo de granulação 400 e 600 para obter uma superfície plana em dentina. Os corpos-de-prova foram divididos aleatoriamente em 6 grupos (n=13). Os sistemas adesivos Clearfill SE Bond, AdheSE and One-Up Bond F foram aplicados sobre a dentina de acordo com as instruções dos fabricantes, estando os mesmos refrigerados (4ºC) ou à temperatura ambiente (23ºC), seguido da união da resina composta Herculite XRV formando um cone invertido. Os corpos-de-prova foram armazenados em água destilada a 37ºC por 24 h e submetidos a ensaios de resistência à tração com velocidade de 0,5 mm/min. As médias (em MPa) foram analisadas estatisticamente pelo teste t de Student com nível de significância de 5%. Não houve diferença estatisticamente significante (p>0.05) entre os valores de resistência de união quando os sistemas adesivos encontravam-se refrigerados ou à temperatura ambiente.

Concluiu-se que nenhum efeito adverso foi observado na resistência à tração quando os sistemas adesivos foram utilizados imediatamente após a remoção da armazenagem sob refrigeração.

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