JOURNAL OF APPLIED ORAL SCIENCE
www.fob.usp.br/revista or www.scielo.br/jaos
J Appl Oral Sci. *2005;13(4):377-81*

EFFECTS OF AGING METHODS ON MICROLEAKAGE OF AN ADHESIVE SYSTEM USED AS A SEALANT ON CONTAMINATED SURFACES

EFEITOS DE MÉTODOS PARA ENVELHECIMENTO NA MICROINFILTRAÇÃO DE UM SISTEMA ADESIVO USADO COMO SELANTE EM SUPERFÍCIES CONTAMINADAS

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Received: February 01, 2005 - Modification: May 10, 2005 - Accepted: September 27, 2005

ABSTRACT

This study evaluated the effects of aging methods on the microleakage of an adhesive system used for sealing pit and fissure surfaces. After acid etching, the occlusal surfaces of 60 permanent teeth were contaminated with 1mL of human plasma and then sealed with OptiBond dual cure, activated in the ramp mode (Optilux 501). The sample was then divided into eight experimental and a control groups. Aging was performed for 20 days in 5, 37, 55 and 5-55^{o}C (200 cycles/day) under water contact or not. After subjecting the specimens to the $AgNO_3$ technique, they were sectioned twice. Dye penetration was measured and the mean values were subjected to ANOVA. No group provided hermetic seal against dye penetration, even the control group (without treatment). Significant aging x water interaction effect (p=0.033) was observed. The group without water contact at 5^{o}C (p=0.015) showed the least dye penetration value. The results led to the main conclusions: 1) all groups showed dye penetration, even the control group; 2) storage teeth at 5^{o}C on dry condition provided the lowest degree of dye penetration; 3) in the presence of water, the temperature variations seem not to affect the microleakage values.
Uniterms: Dentin-bonding agents; Dental leakage; Pit and fissures sealants.

RESUMO

Este estudo avaliou os efeitos de métodos para envelhecimento na microinfiltração de um sistema adesivo usado para selar superfícies oclusais. Após o condicionamento com ácido, as superfícies de 60 dentes permanentes foram contaminadas com 1 mL de plasma e seladas com OptiBond dual cure, ativado pelo Optilux 501, no modo rampa. A amostra foi dividida em oito grupos experimentais e um grupo controle ou imediato O envelhecimento dos espécimes foi realizado por 20 dias a 5, 37, 55 e 5-55°C (200 ciclos/dia) em contato com a água ou não. Após submeter os espécimes à técnica de infiltração com $AgNO_3$, eles foram secionados duas vezes e os valores médios da penetração do corante foram tratados por análise de variância. Nenhum grupo apresentou vedamento hermético na interface, nem mesmo o grupo sem envelhecimento (imediato ou controle). O grupo que permaneceu a 5°C sem contato com a água mostrou o menor grau de penetração (p=0,015) e a interação envelhecimento x água foi significante (p=0,033). Os resultados levam às seguintes conclusões: 1) todos os grupos mostram microinfiltração, inclusive o controle; 2) o grupo mantido a 5^{o}C sem contato com a água mostrou os menores valores de microinfiltração; 3) em contato com a água, as variações térmicas parecem não afetar o grau de penetração do corante.
Unitermos: Adesivos dentinários; Infiltração marginal; Selantes de fossas e fissuras.

INTRODUCTION

Whereas clinical trials are the best way to provide valuable information about the performance of dental materials[17], they are expensive and time-consuming. Furthermore, it is likely that the products under investigation might be discontinued by the time the study is published.

Microleakage tests are often relied upon predictors of clinical performance for new generation adhesive-restorative approaches and materials. These tests usually include thermal cycling to simulate intraoral conditions[1,17], which requires exposure of specimens to water baths set at temperatures that resemble those found in the oral cavity. The rationale behind this is that marginal percolation can be caused by differences in the coefficient of thermal expansion between dental tissues and the restorative material. The polymerization shrinkage, poor bonding of restorative materials to tooth tissues and coefficient of thermal expansion are blamed as the main factors for leakage[17]. One concern of the thermal cycling regimen is that the temperature variations between 5 and 55°C do not necessarily mean that this is the temperature variation that causes damages to the bonding interfaces[11].

Apart from thermal stressing, the presence of water has been generally overlooked in microleakage studies. Bond breakdown may be enabled or facilitated by hydrolysis of the bonding agent and its union with tooth tissue[17]. It is widely known that water acts as plasticizer of polymers and reduces the marginal integrity of restorations[2,3] being a stress-raising factor.

Pit and fissure sealing is a successful clinical procedure to prevent caries development and it is routinely indicated for teeth under caries risk[5,12,24,26,28]. Sometimes, the success of this technique is dependent on the application of the material during the teeth erupting stages. Under such clinical conditions the use of rubber dam[6], to reduce the risk of contamination by saliva, crevicular fluid and/or blood is almost impossible[10,15].

Microleakage and partial/total loss of sealants due to salivary contamination are the main concerns regarding their long-term success[15]. Therefore, adhesive systems have been proposed as an intermediate layer between enamel and sealants for minimizing deleterious effects of non detected moisture contamination, and such combination has being analyzed via *in vitro*[15,16,22] and *in vivo*[8] studies.

A more recent approach indicates the sole use of filled adhesive systems for sealing, mainly when contamination is likely to occur[4,13,29]. It was observed that OptiBond dual cure[29] and OptiBond FL[4] showed less microleakage values whether applied on contaminated enamel surfaces or not, compared to two conventional sealants (Delton and Fluroshield) and three adhesive systems (Scotchbond MP, All Bond and SOLO). This result was also maintained even when the specimens were subjected to thermal and/or load cycling[4,29]. To date, no study has separately addressed the effects of water and temperature changes on the marginal sealing, which was the main objective of the present investigation.

Therefore the objective of this study was to analyze the effects of water contact and aging methods on the marginal sealing of an adhesive system used as a sealant for contaminated condition.

MATERIALS AND METHODS

The Ethics Committee at Dental School, University of São Paulo, approved this study. Sixty caries-free extracted human molars and premolars, free of defects/fractures, were cleaned and immersed in a 10% formaldehyde solution for some days. After that they were stored in distilled water for no longer than 4 months. The roots and cervical region of crowns were embedded in acrylic resin and the teeth were pumiced and rinsed thoroughly with water using an ultrasonic cleaner (Thornton, Inpec Electronics, Vinhedo, SP, Brazil).

After surface conditioning with 32% phosphoric acid for 30 seconds (Uni-etch, Bisco Inc, Schaumburg, IL, USA; batch 9900010368), the sample was contaminated with 1 microliter of human plasma (Pharmaceutical School - University of São Paulo) for 30 seconds, followed by material application, as shown in Table 1. The OptiBond dual cure adhesive (SDS Kerr, Orange, CA, USA; batch 910068, 910A31 and 909D92) was light cured with an Optilux 501 curing unit (Demetron Res. Corp.; Danbury, CT, USA) under ramp mode with light

TABLE 1- Composition and application sequence of the adhesive system

Products	Composition	Procedures
Uni Etch (Bisco Inc.)	1. Etch: 32% phosphoric acid with BAC	
OptiBond (Kerr Co.)	2. Primer: HEMA, GPDM, MMEP, ethanol, water, initiators 3. Bonding agent: catalyst: BIS-GMA, HEMA, GPDM base: barium aluminum borosilicate glass, di sodium hexafluorosilicate, fumed silica (48% filler).	a, b, c, d, e, f, g

a - etch (30 s); b – rinse with distilled water (30 s); c – air-dry (15 s); d – contamination with 1mL of human plasma (30 s); e – primer application (brushed for 30 s and air-dry for 5 s at 30 cm distance); f – bonding agent; g – light activation (30 s).

intensity increasing from 100 to 1000 mW/cm^2 in a period of 20s. The light intensity was checked using the own light curing unit radiometer.

The specimens were then allocated into the following groups: 1) thermocycling- 4,000 cycles, 200 per day, at 5 and 55ºC with a dwell time of 60 s in water environment; 2) thermocycling- 4,000 cycles, 200 per day, at 5 and 55ºC with a dwell time of 60 s without water contact; 3) storage in distilled water for 20 days at 5ºC (thermocycling duration time); 4) storage without water contact for 20 days at 5ºC; 5) storage in distilled water for 20 days at 37 ºC; 6) storage without water contact for 20 days at 37ºC; 7) storage in distilled water for 20 days at 55ºC; 8) storage without water contact for 20 days at 55ºC; 9) immediate or control group, with no aging treatment. All specimens, except those from the control group, were maintained immersed in water for 24 h. The experimental design is depicted in Figure 1.

The specimens contact with water was avoided by means of an individual custom tray, fabricated for each tooth from a 0.2 mm vacuum-formed soft plastic (Nite White, Discus Dental, LA, CA, USA). The custom tray was then glued to the acrylic resin surface with cyanoacrylate resin Super Bonder (Henkel Ltda., Itapevi, SP, Brazil), in order to avoid water penetration during the aging method and only allow temperature changes within tooth. In order to confirm if the temperature changed during thermocycling, a thermocouple was placed within the pulp chamber in some teeth and they were subjected to the thermocycling procedure, validating the method employed.

Subsequently, the crowns of the specimens from all groups were coated with three layers of nail varnish, except from the area 1 mm away from the adhesive interface, and immersed in a 50% $AgNO_3$ aqueous solution (pH > 7.0) in darkness for 2 hours. Afterwards, they were rinsed off, immersed in a photo-developing solution and exposed to fluorescent light for 8 hours[20].

Each specimen was sectioned twice, through the sealed fissure system, with a microtome (Labcut 1010, Extec Co., Enfield, MT, USA) in buccal-to-lingual direction generating 4 cross-sections per tooth that were digitized (ScanJet 4c, HP, Palo Alto, CA, USA) to obtain 600dpi TIFF images. On each cross-section the total linear interface between adhesive and enamel in the buccal and lingual slopes, as well as the extent of the dye penetration were measured (mm) with IMAGELAB (image analysis software developed at FOUSP, Brazil) and expressed as a percentage of the total adhesive interface length. For each tooth, the percentages of dye penetration along the cross-section interfaces were averaged.

An unbalanced ANOVA with empty cells was employed to evaluate the effects of the main effects as well as their interaction with respect to the mean value of leakage[18]. The significance level considered was 5%.

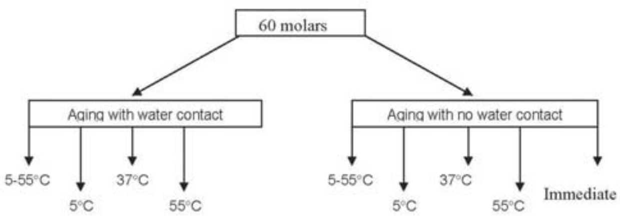

FIGURE 1- Experimental design

RESULTS

Table 2 contains the descriptive measurements per group and Figure 2 shows the mean profile plots with the respective standard errors.

The analysis of variance indicated significant aging methods x water interaction (p=0.033). It is worthwhile to note that the significant water x aging interaction suggests that the differences between the overall dye penetrations for the different methods depend on the storage condition (with water contact or not). The main factors, water (p=0.448) and aging (p=0.186), were not statistically significant.

The Tukey test for multiple comparisons was applied to the different combinations of aging methods and water. Eight of the thirty-six comparisons were statistically significant (considering the level of significance as 5%), five of them corresponding to storage at 5ºC with no water. Results of the Tukey test suggested that the overall dye penetration percentage for teeth stored at 5ºC with no water contact is

TABLE 2- Mean dye penetration and standard errors (%)

Group	Sample size	Average	Standard error
Cycling with water	6	46.7 ^{b,c}	11.5
5ºC with water	6	48.4 ^{b,c}	7.3
37ºC with water	6	24.0 ^{b,a}	9.3
55ºC with water	6	44.7 ^{a,b,c}	14.6
Cycling without water	6	58.8 ^{c}	15.6
5ºC without water	6	15.9 ^{a}	5.2
37ºC without water	6	55.1 ^{c}	9.0
55ºC without water	5	57.7 ^{c}	11.9
Immediate	11	34.6 ^{a,b,c}	7.4

Same superscript letters indicate no statistically significant difference (p>0.05)

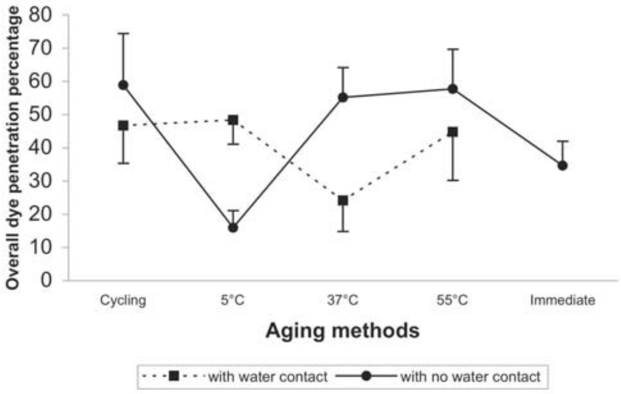

FIGURE 2- Average dye penetration and estimated standard errors (%)

smaller than that for the other methods, which were statistically equivalent. These findings were incorporated into a linear model, which confirmed the above results with statistically significant effect (p=0.015).

Under this approach, the overall dye penetration percentage from the group at 5°C under no water contact was 15.9 ± 11.0 (mean ± standard error). For the other groups, the overall dye penetration percentage was 44.9 ± 3.7.

The measurements were repeated, some time later, for sixteen specimens and the intra-class correlation coefficient was calculated as a measure of reliability; the result was 0.81, indicating excellent agreement[9].

DISCUSSION

The marginal integrity of the tooth/material interface is dependent upon many factors like the physical properties of the material and dental tissue, as well as their interactions with the environment[23]. It is well accepted that a clean, uncontaminated bonding field is mandatory for achieving effective adhesion to tooth structures[5]. The shrinkage that occurs as the material polymerize has been considered the main factor for bonding failures and it may lead to leakage around the restoration and subsequent secondary caries[17]. The linear coefficient of thermal expansion has been noted as other important contributing factor to leakage. However, in a clinical situation, the dimensional change may not be as great as the linear coefficient of the material would predict, once extreme temperatures seem to occur in short periods of time[23]. The clinical relevance of the linear coefficient has not been clarified yet[11] since some time is required for a non uniform temperature body to approach the equilibrium[11,23].

The results of many clinical trials and *in vitro* studies depicted the longevity and effectiveness profile of sealant technique[12,15,24], although there are reports showing decreased retention rates over time[12,15]. The application of hydrophobic materials to acid-etched enamel susceptible to moisture contamination is likely the major cause of reduced retention of fissure sealants[6,7,10,13,15]. Therefore, the use of hydrophilic adhesive systems to seal fissures, followed[15] or not[13] by hydrophobic sealants, is of special interest since they could overcome the negative effects of some casual contamination during the bonding process[6].

As in previous studies no significant differences between microleakage values for dry and contaminated condition were observed when OptiBond was used[4,13,29]. Based on that and in the fact that the use of an adhesive system as sealant is proposed in cases where there is risk of contamination, the authors of the present study decided not to use dry enamel condition.

The present results show that aging at 5°C, without water contact, has an overall dye penetration value of 15.9 ± 11.0%. It is likely that the diffusion coefficient is lowest at 5°C than at 35°C and that the mobility of the polymer molecules are reduced[25], which in turn may restrict the tracer penetration along polymer/enamel interface, since there is no water contact. Although at 5°C in a dry condition lower microleakage was observed, it does not clinically simulate the oral environment. All other experimental groups, except the group maintained at 5°C in a dry condition, showed overall leakages that were statistically similar (44.9 ± 3.7%). This implies that the microleakage degree taken from the control group was statistically similar to the specimens that were thermocycled or stored in one of the aging methods.

For instance, in a previous study[29], the microlekage values of three adhesive systems applied for sealing occlusal surfaces after thermal cycling (4,000 times; 5-55°C) were compared. The authors also kept some specimens in water at 37°C for 72 hours, as control, to verify the interface integrity after a short storage time (to allow possible water sorption and residual polymerization). Under the control condition, dye penetration was observed for all materials regardless the contamination condition; OptiBond showed less overall leakage (11 ± 5%) than the other adhesives (21 ± 6%). After thermal cycling, OptiBond dual cure continued showing the least overall leakage value (28 ± 6%) than All Bond 2 and Scotchbond MP that showed similar values (38 ± 6%). This clearly indicates that microleakage adversely affected early defective interfaces; however, the overall conclusions were not affected by the thermocycling procedures.

As in the study described above, none of the experimental conditions from the present study achieved an initial complete seal. Therefore, it seems that there is no point in subjecting the specimens to thermal cycling. Apart from the thermal changes, other factors such as water sorption may take place and lead to misinterpretation of microleakage results. The effect of water as a polymer plasticizer has been currently overlooked in microleakage studies[11,21] and its effects on thermal cycled specimens should be further evaluated.

The results regarding microleakage coincide with others described in the literature. Neither the storage at a constant temperature or under thermal cycling, nor the dwell period or the number and temperature of cycles seem to significantly affect microleakage[11,14,20]. No difference in microleakage between thermocycled and non-thermocycled groups were detected; breakdown and leakage have also been identified on non-thermocycled specimens interface[19,23,27]. The middle-term storage (3 months) and thermal cycling (500x, 10-60°C) did not enhance the severity of the leakage since the worst values occurred in the immediate group[30].

CONCLUSIONS

Considering the experimental design and the limitations of this study regarding a clinical situation simulating a contaminated sealed surface, it was concluded that: 1) all groups showed dye penetration, even the control group; 2) storage teeth at 5ºC on dry condition provided the lowest degree of dye penetration; 3) in the presence of water, variations seem not to affect the microleakage values.

ACKNOWLEDGMENTS

The authors appreciated the assistance of Dr. Moacir Novelli (LIDO - Informatics Laboratory Dedicated to Dentistry). This study was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, 300086/01-6).

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