Research Article

Effect of decontamination procedures on marginal and internal adaptation in saliva contaminated resin composite restorations

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ABSTRACT

Aim: The aim of this study was to evaluate the efficiency of decontamination techniques on marginal and internal adaptation of resin composite restorations in dentin, after contamination with saliva upon different stages of the application process. Materials and Methods: One hundred and twenty human molars and premolars were randomly distributed into six groups (n=20). Enamel was removed and standardized dentin cavities (diameter:3 mm, depth:2 mm) were filled with an etch-and-rinse one-bottle adhesive and a resin composite under six different surface treatments: (1) adhesive application following manufacturers’ instructions (control); (2) dentin etching, 5-s saliva, 5-s air-dry, adhesive; (3) dentin etching, 5-s saliva, 10-s water-rinse, 5-s air-dry, adhesive; (4) adhesive application/light-cure, 5-s saliva, 5-s air-dry; (5) adhesive application/light-cure, 5-s saliva, 10-s water-rinse, 5-s air-dry; (6) adhesive application/light-cure, 5-s saliva, 10-s water-rinse, 5-s air-dry, adhesive reapplication. Adaptation was evaluated at the upper surface (marginal) and at two consecutive depth levels of 0.5mm each (internal), by measuring the length of the debonded margins relative to the cavity periphery (%DM) and the width of the maximum gap (MG). Results: Statistically significant differences were determined among groups. Group (1) at the upper surface presented the most satisfactory adaptation, whereas, groups (4) and (5) had the significantly poorest adaptation in all levels. Groups (2), (3), and (6) revealed no statistically significant differences in comparison to group (1).Conclusion: Removal of saliva by air or water after dentin etching as well as after the total application of the adhesive followed by reapplication were proved as efficient decontamination procedures.

Keywords: Saliva, Marginal adaptation, Etch-and-rinse adhesive, Microgap, Contamination

INTRODUCTION

A reliable and stable bond between resin composite and tooth structure is needed in order to achieve an optimal clinical performance. The importance of moisture and contamination control is highlighted throughout the dental literature and all efforts are focused on keeping the adhesive substrate free of oral contaminants such as saliva, blood, intersulcular fluid and handpiece oil. Inadequate isolation of the operating field can possibly lead to consequences such as microleakage, marginal discoloration, postoperative sensitivity, caries and pulpal irritation.[1, 2]

The use of rubber dam is recommended for proper isolation and prevention of cavity contamination. Nevertheless, cavities are often located in areas where it is difficult to achieve proper moisture control. Clinicians encounter difficulties in adequate isolation of the operating field in restorations with gingival or subgingival margins and in pediatric patients with poor cooperation.[3]

The effect of dental tissue contamination on the bond strength obtained by adhesive systems has been early investigated and the results showed that various oral fluids significantly reduce the bond strength. [4]Particularly, contamination by saliva of enamel or/and dentin was evaluated in plenty of vitro studies and its negative effect was confirmed in most of them. [5-14] Several decontamination procedures were also examined concerning their potential to restore the non-contaminated bond status. [15-17] The experimental outcomes present a wide diversity, which ranges from no effect to totally recover of the bond strength. [5, 6, 9-12, 17-23]

A variety of factors, such as the type of the adhesive systems, the stage and duration of the contamination, the certain decontamination treatment of the substrate can explain the phenomenon. [24] In general, the saliva contaminated and decontaminated effects are mostly documented by bond strength tests. Taking into account that only 20% correlation has been detected between bond strength and gap analysis studies, the results obtained for the influence of saliva on adhesion can not reflect the adaptation quality, as well. [25] Regarding the adaptation in restorations performed under saliva contaminated and decontaminated conditions, inadequate research has been conducted. Duarte et al (2005) examined the internal adaptation and Yazici et al (2007) the microleakage in Class V cavities. [7, 8] In vitro assessment of marginal and/or internal adaptation per se, without taking consideration other parameters, is not considered as a predictable clinical outcome; however, it is still suggested among the several indicators that should be examined. [25]

AIM

The aim of this study was to evaluate the efficiency of various decontamination techniques on marginal and internal adaptation of cylindrical resin composite restorations with dentin margins when used an etch-and-rinse one-bottle adhesive, which contaminated with natural saliva upon stages of its application process. The null hypothesis tested was that there is no difference among the decontamination techniques applied in terms of restoring the adaptation in saliva-affected adhesive resin composite restorations.

MATERIALS AND METHODS

The experimental protocol applied has been approved by the Committee of Ethics of the Dental School of Athens, Greece. One hundred and twenty freshly extracted caries-free human molars and premolars were selected and stored at 4o C in 0.5 % chloramine-T containing distilled water. The teeth were examined to reassure absence of cracks, fissures or any type of restoration. Afterwards, they were embedded in epoxy resin (Epofix, Struers, Denmark) and subsequently, occlusal enamel was removed with a low speed diamond saw under copious water cooling (Isomet, Buehler Ltd, Lake Bluff, IL, USA) and exposed dentin was ground wet with a 600 grit SiC paper.

Standardized cylindrical dentin cavities (diameter: 3mm, depth: 2mm) were prepared using a 3mm-diameter diamond bur (Proxxon, Niersdorf, Germany) on a micro-milling machine (MF70 Micro-Miller, Proxxon, Niersdorf, Germany) under copious water cooling. Dentin cavities were examined under 200x magnification to ensure absence of pulp exposure. All cavities were filled with an etch-and-rinse one-bottle adhesive and resin composite (Table 1). The resin composite was placed in a single layer, covered with a mylar strip, pressed against a transparent cover slip and photopolymerized for 40 seconds with a light-curing unit of 850 mW/cm2 light intensity (Elipar Trilight 3M/ESPE, Seefeld, Germany) (Figure 1). The restorations were ground gradually with a series of silicon carbide papers of 320-1000 grit size under wet conditions until exposure of the dentin cavity margins. Inspection was made after removing the surface debris with copious amounts of water.

Fresh natural saliva was collected near the time of contamination, provided by a single donor, after brushing, flossing and chewing a paraffin gum for 5 min prior to saliva collection. Saliva was collected in a sterile beaker and was used immediately. Each time, 15 μl of saliva were collected using a micropipette and applied on the substrate. The saliva was left undisturbed for 5 seconds before each decontamination technique was applied.

Teeth were randomly distributed into six groups of 20 each and a different surface treatment was accordingly applied:

* Group 1: adhesive application following manufacturers’ instructions (control);
* Group 2: dentin etching, 5-s saliva, 5-s air-dry, adhesive;
* Group 3: dentin etching, 5-s saliva, 10-s water-rinse, 5-s air-dry, adhesive;
* Group 4: adhesive application/light-cure, 5-s saliva, 5-s air-dry;
* Group 5: adhesive application/light-cure, 5-s saliva, 10-s water-rinse, 5-s air-dry;
* Group 6: adhesive application/light-cure, 5-s saliva, 10-s water-rinse, 5-s air-dry, adhesive reapplication.

Adaptation was determined at the upper surface and at two consecutive depth levels of 0.5mm each, after gradual grinding of each sample (Figure 1); the dimensions were checked with a digital caliper. The specimens were observed under an optical microscope at 200x magnification and the parameters measured were the length of the debonded margins (DM) relative to the cavity periphery (%DM) and the width of the maximum marginal gap (MG) (Figure 2). The microscope used was ME 600 Eclipse (Nikon-Kogakou, Japan) and the measurements were carried out by image analysis software (Sigma Scan 4, Jandel Scientific, CA, USA). Images were recorded with parallel and crossed polarizers, to differentiate the presence of entrapped debris at the interface which might impose a bias on the results.

Kruskal-Wallis and Mann-Whitney U post-hoc tests were used to statistically compare the %DM and MG values among all the groups and levels, to a significant level of 5%, using IBM SPSS software version 21.0.

RESULTS

Figure 3 shows a representative image of restoration with perfect marginal adaptation while figure 4 shows a representative image of a restoration where the interface presented imperfect adaptation. The defects in all of the specimens exhibited the same pattern, as blister-like voids.

Results from the measurement of the length of the debonded margins relative to the cavity periphery (%DM) and the width of the maximum marginal gap (MG) are summarized in Tables 2 and 3, respectively.

Kruskal-Wallis test revealed statistically significant differences among the six groups, for both parameters and depth levels tested at the 0.05 significance level. Mann-Whitney U multiple comparison tests were used to identify the relations among the experimental groups.

Group 1 was the only one that exhibited excellent marginal adaptation with absolute absence of microgaps at the upper surface of the restoration (level 1). However, no statistically significant differences were revealed between group 1 and each of groups 2, 3 and 6 for %DM and MG values per depth level. Concerning the comparison among groups, no differences were noticed among 2, 3 and 6 for all recordings. Groups 4 and 5 showed statistically the highest %DM and MG values, at all three depth levels, without differences among them.

DISCUSSION

A gap-free restoration is considered as a key factor for the optimal clinical performance of resin composite restorations [26-28]. Despite the fact that there is no threshold gap-size for the occurrence of secondary caries, the presence of marginal defects provides potential pathways for bacteria penetration and as a consequence, jeopardizes longevity and clinical success of adhesive restorations. [29] Any kind of contamination during the adhesion procedure may reduce the optimum adaptation that each bonding agent can provide.

Under the clinical point of view, saliva contact in resin composite restorations commonly occurs either after the etching procedure and before the adhesive application or after the completion of the adhesive placement. Both conditions were investigated in the current study.

In this study, human saliva was chosen as the contaminant, contrary to artificial saliva and substitutes which have been used, in order to achieve clinically relevant conditions. [5, 11] In a study of Neelagiri et al., fresh whole saliva is considered an acceptable material to be used for contaminating testing, versus artificial saliva, which is deprived of macromolecules and may confound the results. [15] In order to standardize the contaminating factor and avoid extreme changes in pH and buffering capacity, saliva was always collected from the same individual, following a typical oral hygiene protocol as described in methods section. Saliva was left undisturbed for 5 seconds prior to the application of any decontamination media, with the hypothesis that this is an average time for the clinician to notice and deal with the contamination. The duration of contamination varies significantly among studies and it is a factor that should be particularly considered when comparing their findings.

In the present study, 2mm in-depth cylindrical cavities were performed because the saliva effect should be tested in restorations with a very high configuration factor (c=5). [30] The resin composite was applied in a single increment since efficient polymerization is obtained up to 2mm depth. Multi-layer technique avoided because high risk of flaws at the interfaces of the layers exists. At the same time, 2mm layer is considered as sufficient for the control of net polymerization shrinkage. [31, 32]

The quality of the adaptation was determined by the extent and width of the gaps at the outer margins and the cavity walls, accordingly. Marginal and internal gaps were evaluated as individual parameters of adaptation because the first is not necessarily similar to the second and the opposite. As an experimental procedure, marginal and internal adaptation is considered to be a laborious and time-consuming technique. It often yields false negative results and requires a large number of specimens in each group because of the high variability of the values. [29, 33] Despite the above limitations, it is thought that results concerning the quality of the tooth-restoration interface in combination with the already available bond strength data will enforce the evidence for the various saliva decontaminated techniques. In the current study, only one adhesive system and resin composite product were used, to avoid interference by numerous variables. As a result, possible differences detected can be attributed only to the different stages of contamination and the decontamination techniques applied.

Direct optical microscopic observation of the specimens was preferred to evaluation of replicas by a scanning electron microscopy. Direct observation allows measurements directly on dentin specimens instead of replicas, providing reliable phase identification and allowing evaluation of the same specimen at different levels. It is a simpler, less time-consuming and less destructive method, which avoids any potential drawbacks of the replica technique.[7]

According to the results of the present study, the null hypothesis was rejected since the decontamination procedures showed non-uniform performance concerning the potential to recover the marginal and internal adaptation obtained under non-contaminated conditions.

Saliva consists 99,6% by water and 0.6% by macromolecules, mainly glycoprotein sugars and amylase. [34] Therefore, once saliva contacts the dentin surface, glycoprotein deposits on the dentin surface and may diffuse into dentin tubules, reducing surface energy and rendering the surface unfavorable for bonding. The latter is possible to inhibit or eliminate the infiltration of hydrophilic monomers during the hybridization process, resulting in reduced bonding effect. [15] On the basis of the bond strength investigations, clear conclusion cannot be obtained. Some have reported that the saliva had no adverse effect on the bonding efficiency of one-bottle adhesive systems [9, 11, 12, 17] whereas others stated the opposite finding. [6, 13, 15] Despite these diversities, it is generally suggested to remove the saliva from the substrate, in order to counter the possible contamination effect.

On the basis of the current study outcomes, in cases where saliva was rinsed with water before the application of the adhesive agent (group 3), the gap parameters measured were not significantly different from the analogues of non-contaminated restorations (control). According to dentin pH measurements, Hiraishi et al (2003) established the hypothesis that water rinsing for 5s after 60s saliva contact can completely remove the contaminant. In the present study, contact time was even lower (5s). [5] Contamination time is considered as a significant contributor factor since bond strength investigation revealed that the longer the time, the lower the adhesion values. A longer duration of saliva contact results in water evaporation and in formation of a thick film of glycoproteins, which is more resistant in decontamination techniques. [35]

Similar results with the washed-out treatment were obtained when the saliva was air-dried for 5s (group 2). Air-drying for such short time may leave a moist surface. The bonding agent used in this study (Excite F) is a hydrophilic product. Water of the remaining saliva may aid the diffusion and subsequent penetration of monomers into the irregularities of demineralized dentin, which facilitates micromechanical interlocking. [19] In addition, Excite F contains ethanol as a solvent which displaces and competes water/moisture. [9, 33] Thus, the presence of saliva film may not hinder the process of adhesion mechanism. Simultaneously, ethanol may denature the glycoprotein of the contaminated surface resulting in cleansing of the substrate. Moist dentin surface is considered as an essential factor for good adhesion in etch-and-rinse one-bottle bonding agents. [36]

Direct correlation between adaptation accomplished in a restoration by an adhesive system and its bond strength value has not been established. However, we should mention that the outcomes of the current study are in the same line as those reported in adhesion studies for etch-and-rinse one-bottle agents. Available data reveal that when saliva contamination occurs after etching of the dentine, no significant differences in shear bond strengths are induced between uncontaminated and saliva contaminated dentin treated by blot, air-blast or water rinsing [9, 19, 21, 37]

Contamination of the polymerized adhesive layer resulted in inferior marginal and internal adaptation regardless saliva air drying or water rinsing (groups 4 and 5). The oxygen-inhibited superficial layer of the adhesive agent can easily adsorb salivary glycoproteins [14] although Hitmi & others, 1999 reported no saliva diffusion after curing of the adhesive. [20] It was hypothesized that under saliva presence, it can act as a barrier that inhibits or lessens the copolymerization of adhesive with the above increment of resin composite, causing flaws at the adhesive-resin composite interface. [14] Water rinsing for 10s is possible to fail in displaying the saliva glycoprotein, which remains on the substrate. In addition, water may partially dissolve the air-inhibited superficial layer and consequently, the necessary copolymerization is eliminated. Furthermore, the residual hydrophilic monomers of Excite F can be easily displayed by water rinsing, a phenomenon which facilitates the deterioration of the adhesive layer and accounts for the inferior adaptation recorded.

These results are consistent with those provided by bond strength tests. Significant decline in bond strength was reported when saliva contamination occurred after light-curing for etch-and-rinse two- and one-step adhesive systems. [14, 20, 22]

It is an interesting finding of the present study that application of the bonding agent on the saliva contaminated/water washed/dried adhesive layer (group 6) yielded similar quality of adaptation to the control restorations. The phenomenon may be explained by the hypothesis of multiple adhesive layer effect. Under reapplication, a second coating of adhesive is formed, which increases the bonding strength of hydrophilic agents. [38]

In the current study, the efficiency of decontamination procedures was evaluated when application of saliva conducted under different stages of the bonding protocol of an etch-and-rinse one-bottle adhesive system. Based on the conclusion provided by bond strength studies, that the efficiency of cleansing media and procedures are strongly attributed to the adhesive generation, the results of this trial cannot be generalized to other categories of adhesives. In addition, despite the encouraging results achieved after some decontamination techniques, we should notably consider the hypothesis that possible saliva deposits, as showed in figure 4, remained either on etched dentin or on adhesive layer, may have a negative effect on the long-last performance of a restoration. Therefore, any kind of contamination of the bonding area should, in principle, be avoided.

CONCLUSIONS

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

* Saliva contamination of etched dentin did not negatively affect marginal and internal adaptation of the etch-and-rinse one-bottle adhesive tested when the saliva was removed by air or water before the application of the adhesive.
* Inferior adaptation was observed when the contamination occurred after the light-curing of the bonding agent.
* Removal of saliva and reapplication of the adhesive restored the adaptation.

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FIGURE LEGENDS

Figure 1. Schematic cross-sectional view of the specimen. Adaptation was evaluated at the upper surface and at two consecutive depth levels of 0.5mm each, after gradual grinding of each sample.

Figure 2. Schematic occlusal view of the specimen. Marginal and internal adaptation was evaluated by measuring the length of the debonded margins (DM) relative to the cavity periphery (% DM) and the width of the maximum marginal gap (MG).

Figure 3: Restoration with perfect marginal adaptation.

Figure 4: Restoration with debonded margins.

100 μm



Table 1: Composition and application mode of the adhesive system and resin-composite used.

|  |  |  |
| --- | --- | --- |
|  | Composition | Application mode |
| Adhesive  ExciTE F  (Ivoclar Vivadent, Schaan, Liechnstein) | Phosphonic acid acrylate, HEMA, dimethacrylate, highly dispersed silicone dioxide, initiators, stabilizers and potassium fluoride, ethanol | Phosphoric acid gel was applied on dentin for 10–15 seconds and removed with a vigorous water spray for at least 5 seconds. Excess moisture was removed with an air gun leaving the dentin surface with a glossy wet appearance (wet bonding).  Dentin was saturated with a generous amount of ExciTE F for at least 10 second and excess was removed with a weak stream of air, leaving a uniform, glossy appearance. ExciTE F was light-cured for 10 seconds at 850 mW/cm2. |
| Resin-composite  Tetric EvoCeram (Ivoclar Vivadent, Schaan, Liechnstein) | Bis-GMA, urethane dimethacrylate, ethoxylated Bis-EMA, barium glass filler, ytterbiumtrifluoride, mixed oxide, prepolymers, additives, catalysts and stabilizers, pigments | Resin-composite was placed in a single layer and photopolymerized for 40 s at 850 mW/cm2 light intensity. |

Table 2: Mean values and SD of the %DM parameter

for all groups and depth levels\*.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Group | | DM-level1 | DM-level2 | DM-level3 |
| Group 1  (Control) | Mean | 0.00e,f | 0.02a,e | 0.01d,e |
| SD | 0.00 | 0.04 | 0.03 |
| Group 2 | Mean | 0.01a,b | 0.02b,f | 0.02a,f |
| SD | 0.02 | 0.04 | 0.04 |
| Group 3 | Mean | 0.01c,d | 0.03c | 0.02g.h |
| SD | 0.03 | 0.06 | 0.04 |
| Group 4 | Mean | 0.05a,c,e | 0.06a,b | 0.08b,d,f,g |
| SD | 0.06 | 0.08 | 0.08 |
| Group 5 | Mean | 0.06b,d,f | 0.08c,d,e,f | 0.07a,c,e,h |
| SD | 0.07 | 0.08 | 0.08 |
| Group 6 | Mean | 0.02a | 0.03d | 0.03b,c |
| SD | 0.04 | 0.05 | 0.05 |
|  |  |  |  |  |

\**Same letters within the columns indicate statistically significant differences among the Groups (1 to 6) for each level/column (DM1, DM2, DM3), according to post-hoc analysis, Mann-Whitney U test, p<0.05.*

Table 3: Mean values and SD of the MG parameter for all groups and depth levels \*.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Group | | MG-level1 | MG-level2 | MG-level3 |
| Group 1  (Control) | Mean | 0.00e,f | 5.26a,d | 3.59c,d |
| SD | 0.00 | 13.06 | 9.42 |
| Group 2 | Mean | 4.49a,b | 17.05e | 15.40 |
| SD | 11.65 | 51.81 | 24.91 |
| Group 3 | Mean | 4.92c,d | 15.31b | 6.68e,f |
| SD | 12.51 | 26.89 | 13.95 |
| Group 4 | Mean | 29.43a,c,e | 29.89a | 27.89a,c,e |
| SD | 38.86 | 40.44 | 24.88 |
| Group 5 | Mean | 24.10b,d,f | 30.74b,c,d,e | 29.34b,d,f |
| SD | 28.17 | 26.81 | 26.39 |
| Group 6 | Mean | 11.70 | 12.07c | 10.52a,b |
| SD | 19.11 | 19.97 | 20.32 |

\**Same letters within the columns indicate statistically significant differences among the Groups (1 to 6) for each level/column (MG1, MG2, MG3), according to post-hoc analysis, Mann-Whitney U test, p<0.05.*