Effect of Precuring and Postcuring of Total-Etch and Self-Etch Bonding Agents on the Microleakage of Fissure Sealants

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ABSTRACT

Objectives: Considering the importance of timesaving in pediatric dentistry, if the efficacy is achieved along with shorter working time and less technical sensitivity, the behavior management of young patients can be anticipated. This study aimed to compare the effect of precuring and postcuring of total-etch and self-etch bonding agents on the microleakage of sealants.

Materials and Methods: This study was conducted on forty impacted third molars, which were surgically extracted. The samples were divided into five groups: 1. Control (etching and sealant), 2. Precured fifth-generation bonding agent (Adper Single Bond 2) and fissure sealant, 3. Postcured fifth-generation bonding agent (Adper Single Bond 2) and fissure sealant, 4. Precured seventh-generation bonding agent (Single Bond Universal) and fissure sealant, and 5. Postcured seventh-generation bonding agent (Single Bond Universal) and fissure sealant. All specimens were thermocycled (×1000), immersed in 0.5% basic fuchsine solution for 24 hours, sectioned, digitally photographed, and measured using the LAS EZ program. The data were analyzed using Kruskal-Wallis and Dunn’s tests.

Results: Leakage in the control group and the third group was significantly lower than that in other groups. In pairwise comparisons, a significant difference was found between the control group and the fifth group and between the third group and the fifth group.

Conclusion: The conventional method of sealant placement showed superior results in comparison with the use of an intermediate layer of the bonding agent.

Keywords: Pit and Fissure Sealants; Pediatric Dentistry; Preventive Dentistry

INTRODUCTION

Dental caries is one of the most common diseases that can result in acute infection and tooth loss [1]. In recent decades, a great reduction in the prevalence and severity of dental caries has occurred, which can be attributed to fluoride exposure, increased knowledge of the importance of primary care, and accessibility of dental care units [2]. Although the overall smooth surface caries rate has significantly decreased, the percentage of total caries in pits and fissures...
has increased and accounts for about 80% of all caries in young patients in the United States [3]. One reason for the greater prevalence of carious lesions in occlusal surfaces is that the bristle of a toothbrush has a larger diameter than the width of the fissure; therefore, this surface cannot be thoroughly cleaned [4]. The use of pit and fissure sealants is the most effective means for reducing occlusal caries [5]. A fissure sealant is a material placed over the occlusal surface of the tooth, which penetrates deep pit and fissures. This material presents a protective barrier of the tooth surface and deprives bacteria of nutrients [6].

Several studies have documented the advantages of associating an adhesive system with resin sealants to improve the efficacy of sealant placement. The use of a bonding agent, even on a saliva-contaminated tooth surface, has shown reduced microleakage values [7]. Although the use of a bonding agent has some disadvantages, such as increased working time and increased cost of sealant placement, the use of new bonding agents has improved the retention and bond strength of the sealant material and has reduced microleakage values [8-10]. The bonding procedure in total-etch systems usually has two or three steps. In a three-step system, acid-etching, priming, and bonding procedures are performed separately. In a simpler two-step system, priming and bonding steps are performed simultaneously. The selective dissolution of hydroxyapatite crystals through acid-etching is followed by polymerization of the resin sealant that has penetrated the resultant porosities by capillary infiltration [11]. In self-etch systems, a separate acid-etching procedure is not necessary. This will reduce the clinical working time and the technique sensitivity of the procedure. Self-etch systems can have one or two steps. The self-etch effect is attributed to the monomers that have one or multiple carboxylic or acid phosphate groups [12]. The adhesive agent is commonly cured before the placement of the restorative material [13]. Precuring the adhesive before the placement of the restorative material can lead to the formation of a homogenous hybrid layer, which acts as an elastic cavity wall and relieves the stress produced by polymerization shrinkage [14]. In the postcured (cocured) technique, the adhesive is cured concurrently with the restorative material [13]. The application time is decreased in the postcuring technique, which is merit in treating young children. On the other hand, simultaneous curing of the adhesive and the restorative material may result in insufficient curing of the adhesive layer, and the resultant bond strength may not withstand the stress generated during polymerization shrinkage [13]. On the contrary, some investigators believe that if the thickness of the restorative material is less than two millimeters (mm), lacking the oxygen-inhibited layer in postcured samples can lead to better polymerization and improved bond strength of the adhesive agent [15-17]. Placement of a filled composite material over the uncured bonding agent may result in further polymerization by diffusion of reactive components into the bonding layer [18]. Microleakage is defined as the leakage of microorganisms and toxins through the interface between the restoration and the walls of the cavity [19]. Although microleakage can remain clinically unrecognized, it is one of the most important factors affecting the durability of the restorations. Microleakage can also be related to tooth sensitivity and dental caries [20].

As a result, microleakage assessment is of utmost importance in evaluating a restorative dental material. Considering the importance of timesaving in pediatric dentistry, if the efficiency is achieved along with shorter working time and technical sensitivity, the behavior management of young patients can be anticipated. This study aimed to compare the effect of precuring and postcuring of total-etch and self-etch bonding agents on the microleakage of sealants.

MATERIALS AND METHODS
This in-vitro study has been approved by the ethics committee of the related institute. Using one-way analysis of variance (ANOVA) Power Analysis and Minitab software (Minitab, LLC, State College, PA, USA), the minimum number
of samples in each group was estimated to be 20 sections. Forty impacted third molars were surgically removed according to the treatment plan of an oral and maxillofacial surgeon. The samples were stored in normal saline and transferred into a 1% chloramine-T solution one week before the procedures. An ultrasonic unit was used for the final cleaning of the samples. The occlusal surfaces were examined at ×10 magnification under a microscope to discard those with visible structural defects.

In this study, Scotchbond Universal Etchant (3M ESPE, St. Paul, MN, USA) was used as the etchant. Adper Single Bond 2 (3M ESPE, St. Paul, MN, USA) was used as the fifth-generation (total-etch) bonding agent. Single Bond Universal (3M ESPE, St. Paul, MN, USA) was used as the seventh-generation (self-etch) bonding agent, and Clinpro sealant (3M ESPE, St. Paul, MN, USA) was used as the sealant material.

The operator randomly distributed the teeth into five groups, and the materials were applied according to the manufacturer’s instructions as follows:

Group 1 (the control): the samples were acid-etched with 35% phosphoric acid for 15 seconds and rinsed for 10 seconds. The tooth surface was dried with an air syringe until a frosty enamel surface was obtained. The fissure sealant was placed and light-cured for 20 seconds.

Group 2 (precured fifth-generation bonding agent and fissure sealant): etching was done similar to that of the control group. Two layers of Adper Single Bond 2 were applied and gently air-dried for 5 seconds. The adhesive layer was cured for 10 seconds. Finally, the Clinpro sealant was applied and cured for 20 seconds.

Group 3 (postcured fifth-generation bonding agent and fissure sealant): etching was done similar to that of the control group. Two layers of Adper Single Bond 2 were applied and gently air-dried for 5 seconds. The adhesive layer was cured for 10 seconds. The Clinpro sealant was applied, and both the adhesive and the fissure sealant material were cured simultaneously for 20 seconds.

Group 4 (precured seventh-generation bonding agent and fissure sealant): Single Bond Universal was applied on the tooth surface and rubbed for 20 seconds. The adhesive layer was gently air-dried for 5 seconds, and then, it was cured for 10 seconds. Finally, the Clinpro sealant was applied and cured for 20 seconds.

Group 5 (postcured seventh-generation bonding agent and fissure sealant): Single Bond Universal was applied on the tooth surface and rubbed for 20 seconds. The adhesive layer was gently air-dried for 5 seconds. The Clinpro sealant was applied, and both the adhesive and the fissure sealant material were cured simultaneously for 20 seconds.

All specimens were subjected to thermocycling (×1000, 5±2°C to 55±2°C with a dwell time of 15 seconds and transfer time of 10 seconds). Microleakage was assessed with the dye-penetration method. The apices were sealed with sticky wax, and the samples were coated with two layers of nail polish up to one millimeter from the sealant margins. The samples were immersed in 0.5% basic fuchsin solution and incubated at 37°C for 24 hours.

After rinsing with distilled water, the samples were embedded in epoxy resin. Parallel longitudinal cuts were made in the buccolingual direction using a high-speed diamond saw (Mecatome, T201A, Persi, France) to make samples of 2-mm thickness. A digital image of each section was obtained at ×10 magnification under a stereomicroscope (EZ4D; Leica, Olympus, Tokyo, Japan). The LAS EZ image analysis software (version 1.6.0; Leica Microsystems GmbH, Wetzlar, Germany) was used to measure the extent of dye penetration at the enamel/sealant interface. The microleakage value for each section was calculated by dividing the sum of buccal and lingual dye penetrated surfaces (mm) by the sum of buccal and lingual enamel/sealant interface areas (mm).

A blinded operator made the measurements. The microleakage value was calculated as the mean±standard deviation (SD). Statistical analysis was performed using Kruskal-Wallis test, and pairwise comparisons were made using Dunn’s test. SPSS software (version 23; SPSS Inc., Chicago, IL, USA) was used for data analysis, and the level of significance was set at α=0.05.
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RESULTS

According to the data presented in Table 1, the results of pairwise comparisons based on Dunn’s test (Table 2), and the boxplot of data (Fig. 1), the amount of leakage in the control group and the postcured fifth-generation bonding agent group (the third group) was significantly lower than that of other groups (P<0.05).

In pairwise comparisons, a significant difference was found between the control group and the postcured seventh-generation bonding agent group (the fifth group) and between the postcured fifth-generation bonding agent group (the third group) and the postcured seventh-generation bonding agent group (the fifth group).

DISCUSSION

Microleakage tests are designed to evaluate the sealing capacity of adhesive systems [21]. The dye penetration method is one of the most common techniques used in evaluating microleakage.

In this study, a quantitative method of evaluation was performed. An advantage of this method in comparison with the conventional qualitative scoring is that there is no need for various evaluators, and the scores are distinct [10]. In this section, the effects of adhesive types and curing modes are discussed separately.

The use of bonding agents:

Bonding agents were used to enhance the quality of bonding of the sealant material to the tooth surface [22]. Dental adhesives can be

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Table 1. Descriptive values of microleakage of the five study groups (N=20)

| Groups       | Min  | Max  | Mean | SD   |
|--------------|------|------|------|------|
| Control      | 0.00 | 0.10 | 0.007| 0.024|
| Pre5sealant  | 0.00 | 0.59 | 0.125| 0.186|
| Post5sealant | 0.00 | 0.12 | 0.013| 0.033|
| Pre7sealant  | 0.00 | 0.63 | 0.154| 0.226|
| Post7sealant | 0.00 | 1.00 | 0.317| 0.387|

Min: Minimum; Max: Maximum; SD: Standard Deviation
Table 2. The results of Dunn multiple comparison tests of microleages between the study groups.

| Sample1-Sample2* | Test Statistic | Adjusted Significance |
|------------------|----------------|-----------------------|
| Control-post5sealant | -1.95 | 1 |
| Control-pre5sealant | -15.950 | 0.357 |
| Control-pre7sealant | -18.625 | 0.142 |
| Control-post7sealant | -25.1 | 0.01 |
| Post5sealant-pre5sealant | 14 | 0.653 |
| Post5sealant-pre7sealant | -16.675 | 0.281 |
| Post5sealant-post7sealant | -23.15 | 0.023 |
| Pre5sealant-pre7sealant | -2.675 | 1 |
| Pre5sealant-post7sealant | -9.15 | 1 |
| Pre7sealant-post7sealant | -6.475 | 1 |

categorized as total-etch or self-etch systems. The total-etch technique has been the most widely accepted approach in providing an adequate bond to the enamel. The acid-etching procedure is performed with 30-50% phosphoric acid, which is capable of producing microscopic porosities. This process is followed by capillary infiltration of the adhesive agent and further polymerization [23]. In a study of microleakage and sealant infiltration after using a bonding agent, the control group (acid etching+sealant) showed significantly greater microleakage values [24]. It was concluded that a higher diffusion coefficient of bonding agents and low molecular weight of its monomers can result in better penetration of resin sealants into deep pit and fissures on the occlusal surface [24]. The results of our study were not similar to those reported by Meller et al [24]. This can be justified by different materials and methods of these studies. On the other hand, a clinical split-mouth study found no significant difference in marginal integrity, margin discoloration, and anatomic form whether a total-etch bonding agent was applied under the resin sealant or not [25]. Accordingly, the placement of a bonding agent under sealants did not significantly affect the clinical success of sealants [25]. Similarly, based on the results of the present in-vitro study, the use of bonding agents under fissure sealants did not add significant benefit in comparison with the conventional method. Self-etch adhesives were introduced to simplify the bonding procedure. These agents have acidic monomers, solvents, light initiators, and water, which can simultaneously perform the etching process and penetration of the adhesive agent; they are also less technique-sensitive compared to total-etch counterparts [26]. In a microleakage study of fissure sealants placed on precured total-etch or self-etch bonding agents, no significant difference was found between the study groups [27]. Similarly, in the present study, no significant difference could be found between the precured total-etch and precured self-etch groups. Hannig et al [28] and Celiberti and Lussi [29] found better sealing results in their control samples compared to the sealants applied on self-etch adhesives. According to the results of the present study, the control group had better microleakage values in comparison with the total-etch or self-etch groups. This can be related to higher polymerization shrinkage of adhesive agents compared to resin sealants, which may compromise the bonding quality of enamel/sealant. This can be especially true for self-etch adhesives in which no preliminary
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etching procedure is performed, and the weaker acidic components of these agents are inefficient in demineralizing the tooth structure for achieving a strong bond.

The effect of curing modes:
An oxygen-inhibited layer containing unreacted monomers and oligomers remain after polymerization of self-cure or light-cure resin composites where there is an air interface with the resin. It has been always believed that the presence of this layer is necessary when carrying out an incremental placement procedure [30]. Although in some studies a better bond strength was achieved with the presence of an oxygen-inhibited layer [31,32], some researchers do not support this idea [18,33-37]. After polymerization of the resin material, degradation of the light initiator (camphorquinone) in the oxygen-inhibited layer is claimed to be related to a lower degree of conversion in this layer, and this will interfere with achieving an adequately strong bond to the following layer of the resin material [30]. Microleakage of precured or postcured self-etch and total-etch bonding agents in resin composite restorations placed over primary dentin was studied by Lim et al [14]. Only one postcured total-etch adhesive sample showed some degrees of microleakage, which were not statistically significant. Besides, the depth of penetration of resin tags was higher in the postcured group in comparison with the precured group. In an in-vitro study by Heidari et al [38], the effect of time and light-curing mode on the microleakage of preventive resin restorations was studied. A total-etch bonding agent was used either precured or postcured. According to the results, postcuring resulted in more microleakage, and they suggested that if this technique is preferred to be used, longer curing times should be anticipated. Limited depth of curing in case of precuring the bonding agent was claimed to provide better results [38]. In the present study, the limited thickness of fissure sealant material is not a concern for curing disturbances. The different methodologies of these studies may be the reason for the difference found in the results.

In this study, postcuring the fifth-generation bonding agent (Adper Single Bond 2) and precuring the seventh-generation bonding agent (Single Bond Universal) resulted in better microleakage values. In the total-etch group, lack of an oxygen-inhibited layer in postcured samples (if the thickness of the restorative material is no more than 2 mm) may lead to improved polymerization and better bond strength [15-17]. On the other hand, the placement of a resin composite on an uncured bonding agent may result in further polymerization by diffusion of reactive components [18]. In the one-bottle self-etch group, water has been added to the structure of the adhesive agent to induce chemical reactions with methacrylate acidic monomers to achieve better penetration into the tooth structure [11]. The residual water may prevent the polymerization process of adhesive monomers [39,40]. In postcured self-etch adhesives, the presence of water molecules in uncured bonding layers may interfere with further polymerization of the resin material while in precured self-etch adhesives, water molecules can be entrapped in the cured layer of the adhesive agent and reduce the possibility of interference with the polymerization process.

CONCLUSION
According to the findings of this study, the conventional technique of sealant placement may be appropriate when treating a cooperative young patient, whose cooperation is not expected to be compromised by etching and rinsing procedures. For an uncooperative patient, precuring the self-etch adhesive before the placement of the resin sealant may save time because of the simplicity of the method and the omission of a separate etching phase.

CONFLICT OF INTEREST STATEMENT
None declared.

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