# Impact of adhesive and photoactivation method on sealant integrity and polymer network formation

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Abstract: We evaluated the influence of photoactivation method and hydrophobic resin (HR) application on the marginal and internal adaptation, hardness (KHN), and crosslink density (CLD) of a resin-based fissure sealant. Model fissures were created in bovine enamel fragments (n = 10) and sealed using one of the following protocols: no adhesive system + photoactivation of the sealant using continuous light (CL), no adhesive system + photoactivation of the sealant using the soft-start method (SS), HR + CL, or HR + SS. Marginal and internal gaps and KHN were assessed after storage in water for 24 h. The CLD was indirectly assessed by repeating the KHN measurement after 24 h of immersion in 100% ethanol. There was no difference among the samples with regard to marginal or internal adaptation. The KHN and CLD were similar for samples cured using either photoactivation method. Use of a hydrophobic resin prior to placement of fissure sealants and curing the sealant using the soft-start method may not provide any positive influence on integrity or crosslink density.

Descriptors: Hardness Tests; Dental Marginal Adaptation; Polymerization.

Declaration of Interests: The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

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Received for publication on Oct 25, 2011
Accepted for publication on Feb 14, 2012

## Introduction

Dental decay remains a common disease of the oral cavity.[1] The efficacy of fissure sealants on the primary and secondary prevention of caries disease has been well demonstrated.[2,3] However, their efficacy relies directly on their ability to thoroughly fill pits and fissures and remain intact and bonded to the enamel surface for a lifetime.[4,5] Therefore, factors which may affect marginal adaptation of the sealant must be studied.

A well-adapted sealant and a strong polymeric network are required for sealant effectiveness.[6] Although some authors have suggested that application of adhesive systems can improve marginal adaptation and retention of fissure sealants,[7] others have argued that this procedure offers no benefits to sealant retention or marginal integrity.[8] Complete retention of sealant material in the fissures depends on adhesion to enamel,[9] and the influence of adhesive systems may be predicted by analyzing the superficial and internal margins of fissure sealants.

In an attempt to decrease stresses during polymerization of resin-based materials, alternative photoactivation methods such as soft-starting have been proposed. These methods modify the polymerization kinetics by modulating the power density during photoactivation.[10] The soft-start modulated photoactivation method was reported to be effective in reducing contraction stress and improving the strength of the bonded interface without compromising the polymerization quality of the restorative composite.[11]

Although resin-based fissure sealants possess low elastic moduli to facilitate flow during polymerization, the occlusal surface can present a higher C factor, leading to the development of elevated shrinkage stress.[9] Photoactivation of fissure sealants using soft-start methods might decrease polymerization stresses, improving marginal adaptation. However, the literature is unclear concerning the benefits of soft-start photoactivation on maintaining marginal integrity or the effect on polymer network formation of fissure sealants.

Using a novel fissure model, we aimed to evaluate the use of an adhesive system before sealant placement as well as soft-start modulated photoactivation on superficial and internal adaptation, sealant hardness, and crosslink density. Our hypothesis was that superficial and internal marginal adaptation of the sealant would be improved by the adhesive system and by modulated soft-start photoactivation without decreasing sealant hardness or crosslink density.

# Methodology

## Sample preparation

Commercial brand names, chemical compositions, and lot numbers for the materials used in this study are listed in Table 1. Representative examples of the sample preparation stages are depicted in Figures 1A-I. Forty bovine incisors were used to prepare the samples (Figure 1A). The crowns were sectioned in the mesiodistal direction using double-faced diamond disks (KG Sorensen, Barueri, Brazil) (Figure 1B) in an attempt to obtain 8 mm-long enamel/dentin fragments (Figure 1C). These fragments were then embedded in polystyrene resin (Figure 1D) and the enamel surface was ground on a water-cooled mechanical polisher (Arotec, São Paulo, Brazil) using 320-, 600-, and 1200-grit silicon carbide (SiC) abrasive papers (Figures 1E and F). A cylindrical diamond bur (#1094, KG Sorensen, Barueri, Brazil) was mounted in a high-speed handpiece (Kavo, Joinville, Brazil), positioned at 45° to the enamel surface (Figure 1G), and used to generate a groove in the enamel (Figure 1H) under constant air-water cooling to produce fissure models measuring 1 mm deep × 8 mm long (Figure 1I). To achieve a uniform fissure size, the handpiece was mounted on a cavity standardization device. The diamond bur was replaced after every 5$^{th}$ preparation. The specimens were examined in a stereomicroscope (Carl Zeiss, Manaus, Brazil) at 25× magnification to verify whether the enamel remained on the lower surface.

The materials were prepared according to the manufacturers' recommendations. The fissure was etched using 35% phosphoric acid gel for 15 s, rinsed for 30 s and dried for 30 s with oil-free air. When applicable, a hydrophobic resin was applied after enamel etching and light cured for 10 s using the Ultra-Lume LED 5 (Ultradent, South Jordan, USA). The resin-based fissure sealant was placed in the fissures, covered with a polyester strip, and photoactivated according to the protocol specified for that sample group. The continuous light photoactivation method involved curing the material for 20 s at 800 mW/cm$^2$ (following the manufacturer's instructions), resulting in a total energy of 16 J. The soft-start photoactivation method was standardized with an initial light exposure of 10 s at 150 mW/cm$^2$, followed by 18 s at 800 mW/cm$^2$ (total energy 16 J).

The specimens were stored in distilled water at 37°C for 24 h and then finished and polished in a water-cooled mechanical polisher (Arotec, São Paulo, Brazil) using 600- and 1200-grit SiC sandpaper.

Table 1 - Materials used in this study.

| Commercial brand/manufacturer | Chemical composition | Lot number |
|---|---|---|
| Scotchbond Etchant<br>3M ESPE, St Paul, USA | Phosphoric acid (35%) | 7JL |
| Scotchbond Multi Purpose<br>3M ESPE, St Paul, USA | Adhesive: Bis-GMA$^A$, HEMA$^B$ | 7PY |
| Fluroshield<br>Dentsply/Caulk, Milford, USA | UED-Bis-GMA$^C$; resins; PENTA phosphate; Bis-GMA$^A$; glass filler; silica amorphous; $TiO_2$; NaF | 142812B |

A: bisphenol-A diglycidyl methacrylate; B: hydroxyethyl methacrylate; C: urethane modified Bis-GMA dimethacrylate.

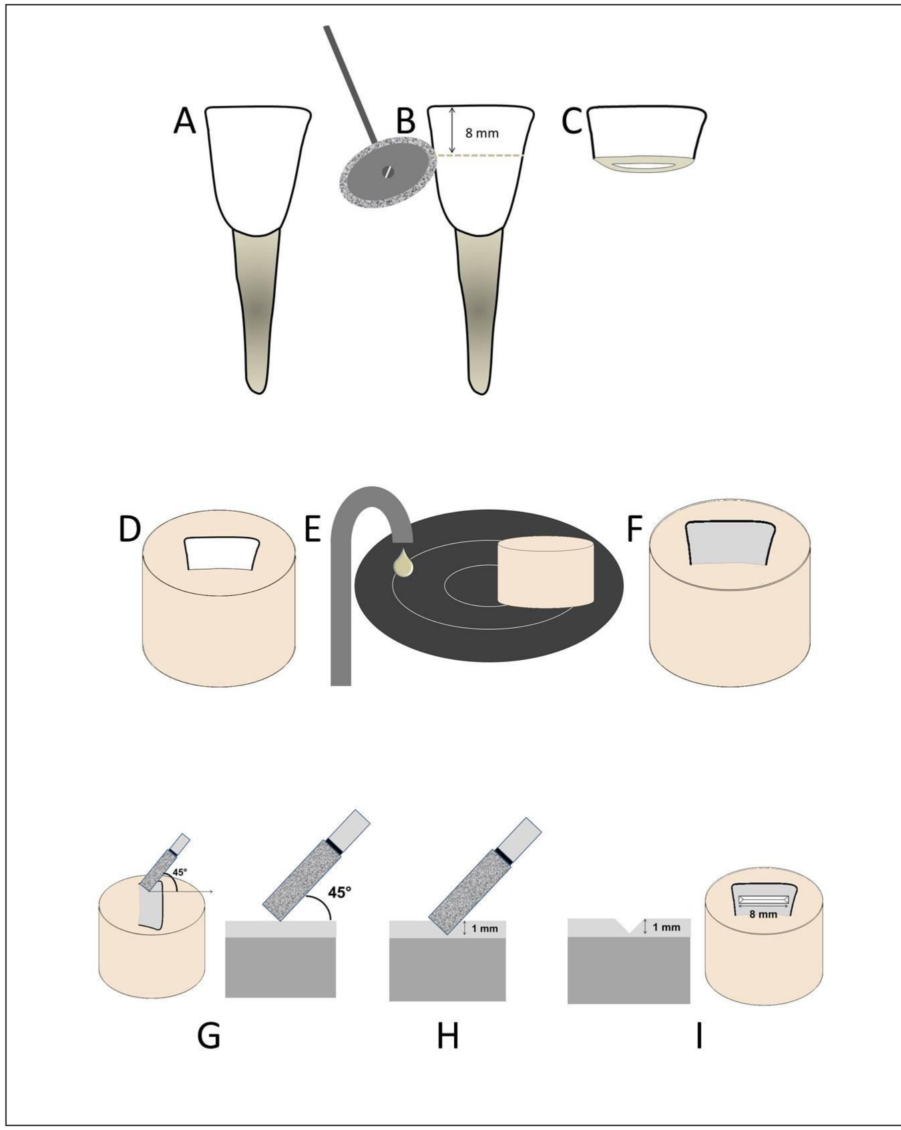


Figure 1 - Sample preparation: lower bovine incisors were used as a substrate (A); the crowns were sectioned in the mesio-distal direction (B) to obtain enamel/dentin fragments (C), which were embedded in polystyrene resin (D); the enamel surface was ground (E) to expose a flat enamel area (F); a diamond bur was positioned on the enamel (G) and passed along (H) the enamel surface to obtain the fissure models (I).

## Marginal adaptation analysis

A 1.0% acid red propylene glycol solution (Caries Detector, Kuraray, Osaka, Japan) was applied at the restoration margins for 5 s. After staining, the specimens were rinsed in tap water and gently blown dry. The fissure margins were evaluated using a Leica MZ6 stereomicroscope (Leica Microsystems, Heerbrugg, Switzerland) at 16× magnification. A digital image of each specimen was obtained at this stage. The lengths of the dye-stained gaps along the fissure margins were measured (in millimeters) using the Image tool software version 2.0 (University of Texas Health and Science Center, San Antonio, USA). The length of the marginal gap was calculated as a percentage of the entire margin length. Samples were also scored according to the presence (score 1) or absence (score 0) of gaps.

## Internal adaptation analysis

Three slices from sealed fissures (1-mm thick) were cut in the mesio-distal direction using an Isomet 1000 machine (Buehler, São Paulo, Brazil). Caries Detector solution was applied to the internal interfaces, and the same procedures described previously were performed for the evaluation of internal adaptation. The internal gap percentages of the slices were averaged to obtain a mean for each specimen. The samples were also scored according to the presence (score 1) or absence (score 0) of gaps.

### Knoop hardness test (KHN)

After analyzing the internal margins, the KHN was evaluated for two slices. An initial hardness ($MH_i$) reading was obtained on the top surface of each specimen using a Knoop hardness tester (HMV-2T E, Shimadzu Corporation, Tokyo, Japan, 50 g load for 15 s). A total of five Knoop measurements were performed on the top surface of each specimen: one at the center and the other four at a distance of approximately 200 µm from the central location. The average of the five values was used to represent the KHN value of each specimen.

### Crosslink density (CLD)

After analysis of KHN, all specimens were immersed in absolute ethanol (100%) at room temperature. The CLD was indirectly estimated based on the percentage decrease in hardness (%HD) that occurred as a result of ethanol exposure.[12] After immersion for 24 h a second hardness reading was obtained ($MH_f$). Five Knoop measurements were performed on the top surface of each specimen as previously described. The results were tabulated and the %HD was calculated using the following equation:

$$\%HD = 100 - [(MH_f \times 100) / MH_i],$$

where $MH_f$ represents the final KHN value (after ethanol storage) and $MH_i$ represents the initial KHN value (before ethanol storage).

### Statistical analysis

Marginal and internal adaptations were determined from the percentage of gaps (quantitative data) and the presence or absence of gaps (qualitative data). To compare the quantitative data, the Kruskall-Wallis test was performed, while the Mann-Whitney test was used to analyze the qualitative data. KHN and %HD were evaluated by means of Analysis of Variance (ANOVA) and Tukey's test. The level of significance was set at $P < 0.05$. The Assistat Beta 7.5 software (Federal University of Campina Grande, Campina Grande, Brazil) was used to perform all tests.

## Results

### Superficial marginal adaptation

There were no differences between groups in terms of percentage of superficial marginal gaps or presence of gaps. The median gaps and gap scores related to superficial marginal adaptation are listed in Table 2.

### Internal marginal adaptation

There were no differences between groups either in percentage of internal marginal gaps or scale rank. The median gaps and gap scores related to superficial marginal adaptation are listed in Table 3.

### HKN and CLD

There were no differences between groups in either initial KNH or %HD (CLD). Means and standard deviations for initial KHN (before ethanol storage), final KHN (after ethanol storage), and percentage hardness decrease are contained in Table 4.

## Discussion

An important parameter for evaluating the clinical success of sealant materials is the marginal adaptation. Many studies[13,14] have used human third molars to evaluate the integrity of fissure sealants. However, the fissure morphology of the human third molar is highly variable,

Table 2 - Median gaps (%) and gap scores related to superficial marginal adaptation.

| Adhesive strategy | Photoactivation method | Median | Scores 0–1 |
|---|---|---|---|
| No adhesive system | CL | 0.0 | 7–3 |
| | SS | 0.0 | 10–0 |
| Application of a hydrophobic resin* | CL | 0.0 | 8–2 |
| | SS | 0.0 | 9–1 |

*Hydrophobic resin of Scothbond Multipurpose Plus. CL: continuous light; SS: soft-start.

Table 3 - Median gaps and gap scores related to internal marginal adaptation.

| Adhesive strategy | Photoactivation method | Median | Scores 0 – 1 |
|---|---|---|---|
| No adhesive system | CL | 0.0 | 18–2 |
| | SS | 0.0 | 18–2 |
| Application of a hydrophobic resin* | CL | 0.0 | 19–1 |
| | SS | 0.0 | 19–1 |

*Hydrophobic resin of Scothbond Multipurpose Plus. CL: continuous light; SS: soft-start.

Table 4 - Means and standard deviations for initial KHN, final KHN, and percentage hardness decrease (%HD).

| Adhesive strategy | Photoactivation method | initial KNH ± sd | final KNH ± sd | %HD ± sd |
|---|---|---|---|---|
| No adhesive system | CL | 26.6 ± 1.5 | 17.4 ± 1.4 | 34.5 ± 6.5 |
| | SS | 26.2 ± 2.3 | 17.1 ± 1.0 | 34.6 ± 5.3 |
| Application of a hydrophobic resin* | CL | 26.0 ± 4.1 | 17.0 ± 2.8 | 33.9 ± 7.6 |
| | SS | 25.7 ± 4.3 | 16.7 ± 2.6 | 33.9 ± 6.8 |

*Hydrophobic resin of Scothbond Multipurpose Plus adhesive system. CL: continuous light; SS: soft-start.

and does not permit standardization of the C-factor. For this reason we employed an artificial fissure model to provide similar conditions while avoiding the influence of the C-factor.

Clinically, sealants are applied to fissures with intact, aprismatic enamel instead of the ground, prismatic enamel[15] present in the fissure model used in this investigation. However, some authors[16,17] have reported no difference in the adhesion of etch-and-rinse adhesive systems to ground or unground enamel. The use of ground, prismatic tissue in this study most likely did not influence the results since an etch-and-rinse adhesive system was utilized. The preparation of simulated fissures in bovine enamel to evaluate the integrity of fissure sealants has the advantage of standardizing the C-factor of the fissure using an acceptable substitute for human teeth.

In the present investigation the marginal and internal gap occurrence was assessed by staining the fissure margins with a 1.0% acid red propylene glycol solution. This technique has been effectively used to evaluate gap formation, but not microleakage.[18-21] A flat, finished, and polished specimen is required to perform this technique without bias, and the samples were flattened, finished, and polished using abrasive paper even though this procedure is not commonly performed *in vivo*.

No benefits were achieved in terms of early sealant integrity by applying a hydrophobic resin or curing the fissure sealant using the soft-start method, although this photoactivation method did not decreased hardness or CLD. Therefore, our hypothesis was partially validated. As the marginal and internal integrity was assessed 24 hours after polymerization, the presence of gaps could be attributed exclusively to polymerization shrinkage. The monomer conversion resulted in similar stresses during photoactivation of the resin-based fissure sealant. This might have been facilitated by the flow characteristics of the material providing better accommodation in the filled substrate due to its low viscosity. On the other hand, if the samples had been subjected to aging conditions simulating an oral environment, it is likely that some differences might be observed. Further studies must be conducted to evaluate the long-term efficacy of the soft-start method.

Applying a hydrophobic resin before sealant placement did not improve sealant integrity, possibly because sealants have reduced surface tension and flow characteristics that promote satisfactory adhesion to the enamel after acid etching. Although application of an adhesive system prior to the sealant has demonstrated a positive effect on microleakage,[21] several criticisms of this method have appeared due to its low reliability in predicting the true infiltration of substances.[22] Tracers used in microleakage investigations are generally not suitable for testing the marginal seal of restorations *in vitro*, as the molecules are so small that they penetrate through tiny and invisible paths along the substrate/restorative material interface. Evaluating the marginal integrity as performed in this work represents a more realistic method.[23]

Considering the absence of negative effects for modulated photoactivation methods such as the soft-start method on select physical properties of resin-based materials, the results of this study corroborate those found elsewhere with respect to degree of conversion[24] and crosslink density.[25] In fact, composite materials submitted to equivalent radiant exposure irrespective of the curing method possess similar degrees of conversion.[24] Although other photoactivation methods such as the pulse-delay can decrease the crosslink density of polymeric networks, the soft-start method did not reduce the level of crosslinking.[25] It must be pointed out that insufficient crosslinking of the polymer matrix may make resin-based materials more sensitive to the plasticizing effects of exogenous substances containing chemicals such as acids, bases, salts, alcohols, and oxygen that enter the oral environment during eating and drinking[25] and may have a deleterious effect on the polymer network and compromise its clinical efficacy.

## Conclusion

The use of a modulated photoactivation method such as the soft-start and a hydrophobic resin before sealant placement did not provide any early improvement in marginal or internal adaptation of the pit and fissure sealant tested. Hardness and crosslink density were not affected by the photoactivation method.

## Acknowledgements

The authors thank KG Sorensen (Barueri, Brazil) for providing some of the materials used in this study.

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