Scientific Article

Effect of Preparation Technique, Fissure Morphology, and Material Characteristics on the In Vitro Margin Permeability and Penetrability of Pit and Fissure Sealants

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Abstract: ***Purpose:*** *The purpose of this study was to evaluate the effect of material characteristics, preparation techniques, and fissure morphology on the microleakage and penetrability of pit and fissure sealants.* ***Methods:*** *Sealants used in the study included: (1) Aegis; (2) Conseal f; (3) Admira Seal; (4) Triage; and (5) Delton Opaque. A total of 100 extracted permanent molars were randomly assigned to 10 groups that combined the materials and preparation technique (pumice prophylaxis and air abrasion). Following sealant placement, the specimens were: (1) subjected to thermal-cycling and dye immersion; (2) invested in acrylic; (3) sectioned for microscopic examination; and (4) assessed for dye penetration (microleakage) and penetrability.* ***Results:*** *Significant differences in microleakage were seen. Aegis using pumice-prophylaxis surface pretreatment showed significantly less leakage than the other groups. Significant differences were also exhibited between groupsseen regarding sealant penetrability, with Delton and Triage (pumice prophylaxis) revealing the greatest values. Fissure morphology was not a significant factor regarding microleakage. Morphology did significantly impact sealant penetrability, however, with u-type fissures displaying the greatest values. No correlation was found between the extent of microleakage and sealant penetrability.* ***Conclusion:*** *Material characteristics and fissure morphology were significant factors regarding sealant success, while surface preparation did not play an important role in sealant microleakage or fissure penetrability. (Pediatr Dent 2007;29:308-14)*

KEYWORDS: PIT AND FISSURE SEALANT, MICROLEAKAGE, FISSURE MORPHOLOGY

Prevention of pit and fissure caries has progressed from early treatment modalities. These include mechanical fissure eradication and chemical treatment using silver nitrate to the development of more innovative and progressive materials and methods, such as micromechanical bonding of artificial resins to enamel substrate using acid etchant techniques.[1-3]

Caries rates involving pits and fissures and smooth surfaces have decreased in number and intensity since the 1970s.[4] Research has focused on the prevention of occlusal caries, since caries involving fissured surfaces comprises over 80% of all carious lesions in young permanent teeth, while making up only 13% of total tooth surfaces.[5] A contemporary approach for sealant placement has includes an assessment of teeth judged "at risk for caries" and not necessarily directed to all teeth with pits and fissures.[6]

The term "pit and fissure sealant" has been documented as a "material that is introduced into the occlusal pits and fissures of caries-susceptible teeth, thus forming a micromechanically bonded, protective layer, cutting access of caries-producing bacteria from their source of nutrients."[7] Traditional resin-based sealants (Nuva-Seal, LD Caulk, Milford, Del) were first introduced in 1971, benefiting from Buonocore's[3] original research with enamel surface etching utilizing high concentrations of phosphoric acid.

Marginal microleakage following sealant placement allows bacterial and bacterial byproducts to penetrate beneath the sealant, potentially initiating and perpetuating the caries formation process.[8] Factors affecting the degree of microleakage include: (1) material shrinkage; (2) salivary and debris components; and, possibly (3) lubrication oil from a dental handpiece.[9]

Pumice-prophylaxis surface pretreatment has been the standard for cleaning occlusal pits and fissures prior to sealant application. Different preparation methods, however, such as air abrasion using aluminum oxide particles and surface acid-etching, have been studied, with inconclusive results.[8-20] Also, various drying agents (acetone) and cleaning/bonding agents (self-etch primer/adhesives) to promote increased adherence and decreased marginal leakage have

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been reported.[10,15,17-20]

Several studies have concluded that fissure type is a significant factor in the penetrability of sealants.[8,10,20-22] Deep fissures can possibly: (1) limit the penetrability of preventive restorative materials; (2) complete sealant placement; and (3) successful retention. Pit and fissure internal anatomy can also serve as an ideal location for the growth and propagation of bacteria and, hence, caries progression.

The purpose of this in vitro study was to evaluate the microleakage and penetrability of 5 sealant materials, considering the following variables: (1) tooth surface preparation techniques; (2) fissure morphology; and (3) material characteristics.

Methods

The treatment groups in the present study were defined by the combination of sealant material and preparation technique. For this research protocol, the authors chose newly marketed sealants employing diverse chemical composition, imparting reportedly different physical characteristics. This study also compared surface preparation techniques: (1) a traditional pumice-prophylaxis cleaning regimen; and (2) the somewhat controversial use of air-abrasion. All sealants, including etchants, were used, strictly following manufacturer's instructions. Fissure type was also studied to evaluate the effects of morphology on sealant interface leakage and penetrability.

Material characteristics

The 5 different sealant restoratives included:

1. Admira Seal (Voco, Cuxhaven, Germany) containing an organically modified ceramic (Ormocer) filler component;
2. Conseal f (Southern Dental Industries, Bensenville, Ill), a barium-fluorosilicate filled resin;
3. Triage (GC America, Alsip, Ill) glass ionomer-based material releasing fluoride;
4. Aegis (Boswoth Co, Skokie, Ill) amorphous calcium phosphate (ACP); and
5. Delton Opaque (Dentsply Professional, York, Pa), a 0% filled resin with no fluoride release.

In this institutionally-approved study, a total of 100 extracted, noncarious human permanent first and second molars—free of macroscopic fractures or other defects, fissure sealants, and/or restorations—were carefully cleaned of calculus and other debris. The teeth (maxillary and mandibular) were randomly divided into 10 groups of 10 each (N=100).

For the pumice prophylaxis (PP) prepared occlusal surfaces, all pits and fissures were cleaned for 15 seconds with an oil-free powdered pumice aqueous slurry using a disposable rotating prophylaxis cup with a bristle brush inserted in a slow-speed, contra-angle handpiece. The teeth were rinsed with air-water spray and dried, followed by etchant application, sealant placement, and polymerization with a halogen light for 20 seconds.

Occlusal surfaces prepared by an air-abrasion (AA) unit (Handiblaster, Chameleon Dental Products, Kansas City, Kan) were etched for 15 seconds with 30 µm of aluminum oxide particles at 40 psi. The handpiece orifice was held at a distance of 2.0 to 2.5 mm from the tooth surface. Following air abrasion, the occlusal surfaces were rinsed and dried, followed by sealant placement and light polymerization. A notable exception for material handling occurred with the use of Triage. Following surface preparation techniques, rinsing, and drying procedures, Triage was manipulated into all pits and fissures with a microbrush applicator, followed by light curing for 20 to 40 seconds. GC Fuji Varnish (GC America, Alsip, Ill) was then applied to the treated areas and adjacent surfaces.

All restorative materials were polymerized with a Schein (Sullivan-Schein, Melville, NY) halogen light. The light was continuously monitored with a radiometer and provided with adequate intensity (≥600 mW/cm2). Following sealant placement, the specimens were stored in distilled water at 37°C for 7 days prior to leakage assessment.

Microleakage assessment

The specimens were thermocycled for 1,000 cycles in separate distilled water baths of 5°C and 55°C with a dwell time of 60 seconds in each bath and a transfer time of 3 seconds. The root apices were then sealed with utility wax and 2 coats of commercial nail varnish was applied to the entire tooth surface to within 1 mm of the sealant. The specimens were immersed in 1% methylene blue dye for 24 hours at room temperature to allow dye penetration (if any) into possible gaps between the enamel and sealant. The specimens were: (1) removed from the dye solution; (2) rinsed with tap water; and (3) allowed to dry. The specimens were invested in clear acrylic autopolymerizing resin and labeled. A Buehler Isomet (Buehler Ltd, Evanston, Ill) water cooled high-speed diamond saw produced 2 parallel cuts of the specimen block (sectioned transversely, from buccal to lingual surface), yielding 4 surfaces per tooth available for scoring.

Microscopic examination

Dye penetration (microleakage), sealant penetrability, and fissure morphology were evaluated using a binocular light microscope at a magnification of X40. Two parallel cuts (3 sections) yielded 8 dye penetration readings (buccal and lingual surface per section), for a total of 80 leakage measurements and 4 sealant penetrability readings per tooth or 40 per group (10 tooth specimens). Based on an ordinal ranking system, the degree of microleakage and sealant penetrability was determined (Figure 1). Microleakage assessment was based on a scoring system suggested by Blackwood et al[9] and Overbo and Raadal.[23] Assessment of fissure morphology classification was recommended by Duangthip and Lussi[10,20] (Figure 2).

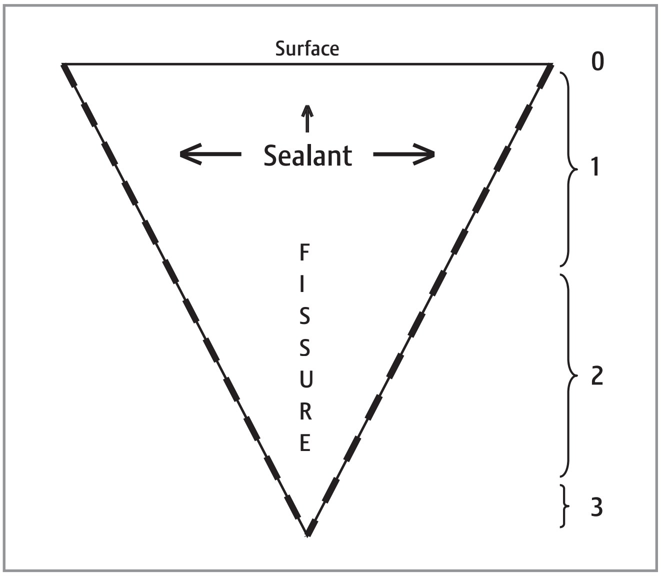

Figure 1. Dye penetration (microleakage) scoring: 0=no penetration; 1= penetration up to one half the sealant's length; 2= penetration greater than one half, not including the underlying fissure; 3= penetration into the underlying fissure. Sealant penetration scoring: 0= no penetration; 1= penetration up to one half the fissure's length; 2= penetration over one half the fissure's length; 3= complete penetration and adaptation into fissure.

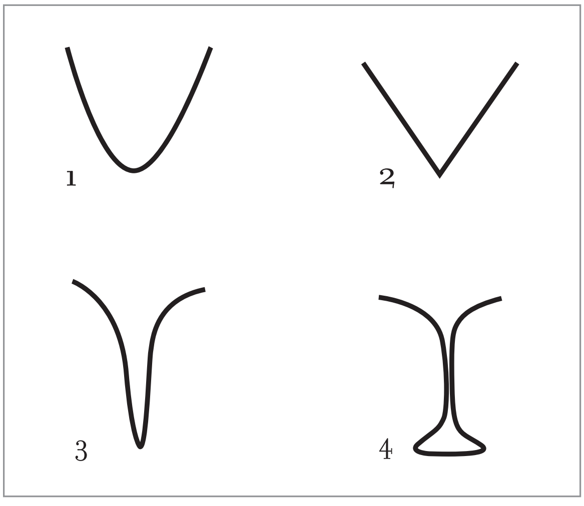

Figure 2. Fissure Morphology Classification: 1=u-type; 2=v-type; 3=y^1-type; 4=y^2-type.

Microleakage, sealant penetrability, and fissure morphology were evaluated by 2 blinded examiners using coded treatment groups. Examiner 1 received training from examiner 2, who had previous experience with microleakage scoring. In the event that disagreement occurred between the 2 examiners during initial evaluation of the specimens, further discussion ensued. The overall score for each tooth equaled the highest score of the 8 potential readings from the buccal and lingual surfaces. The diamond blade was sufficiently thick (0.3 mm) to prevent adjacent surfaces from being "mirror images" of each other. In many instances, 1 surface displayed microleakage, and the adjacent sectioned surface showed no leakage.

Microscopic photographs

Representative microscopic photographs (Figures 3-7) are revealed for each group at either X20 or X40 magnification, showing various stages of sealant microleakage and penetrability together with fissure morphology. A digital camera for microscopy was used for digital image retrieval and downloaded using DirectX 9.0 (Microsoft Corp, Redmond, Wash) and Minisee 1.0 (Hangzhou Scopetek Opto-electric Co, Ltd, Hangzhou, China) software.

Statistical analyses

This study's protocol employed grouping of the sealant material and surface preparation technique into 10 major groups. Three independent variables (material, preparation technique, and fissure type) were examined for individual differences and interactions, with respect to microleakage and penetrability. The Kruskal Wallis nonparametric and, if applicable, Mann-Whitney multiple comparison tests were used for statistical comparison at the $P<.05$ level of significance. Microleakage and sealant penetrability were also subjected to simple regression analyses.

Intra- and interexaminer reliability

A total of 20 specimens (at least 1 specimen from each group) were randomly selected and examined by the 2 examiners under the same conditions and with the same equipment. Intraexaminer reproducibility for examiner 1 was established by repeat examination of selected specimens prior to interexaminer (both examiners) reliability evaluation. Interexaminer reproducibility was analyzed using Cohen's unweighed kappa statistic.

Results

Results from the duplicate examination of the specimens revealed that the interexaminer reliability on microleakage—as assessed by Cohen's kappa statistic—showed "substantial agreement" (0.61).[24,25] Further analysis demonstrated significant agreement between examiners regarding specimen scoring: (1) microleakage (82%); (2) sealant penetrability (83%); and (3) fissure morphology (80%).

Microleakage (specimen-level) analyses

The distribution of microleakage scores for all 100 specimens is shown in Table 1, using the worst section-surface-level (8 total surfaces) leakage score for each specimen. The

Kruskal-Wallis test showed a significant difference between all groups, with Mann-Whitney revealing significant differences comparing paired groups. The Aegis PP group exhibited significantly lower leakage than the majority of the other groups. An intragroup comparison showed the most striking difference exhibited between groups Triage PP and Triage AA.

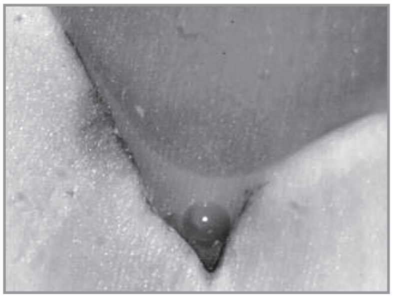

Figure 3. Representative microscopic photograph (X40) of Admira Seal. Note dye penetration (leakage) along buccal (left side) and lingual walls (right side).

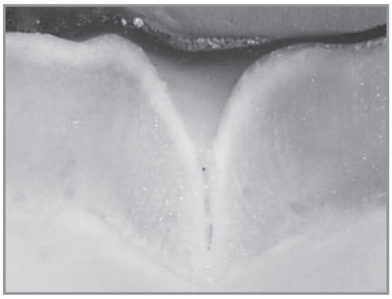

Figure 4. Representative photograph (X20) of Aegis. Note that the leakage along the lingual wall (right side) is believed to be caused from dye absorption by sealant material and not from marginal penetration.

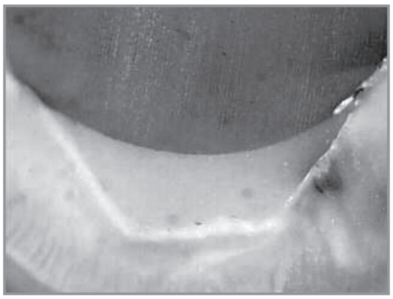

Figure 5. Representative photograph (X20) of leakage (category 1) along the lingual wall (right side) from Conseal f.

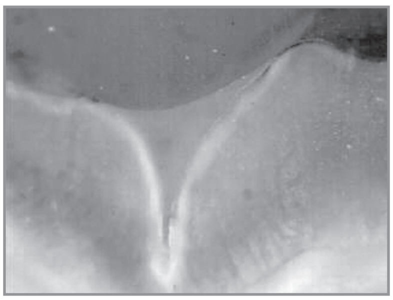

Figure 6. Representative photograph (X20) of Delton showing category 1 leakage along the lingual wall (right side).

Figure 7. Representative photograph (X40) of Triage. Note the problematic characteristics associated with this material: dye absorption, fractures (lingual wall), and leakage (buccal wall).

No leakage (score=0) was measured in 44% of the specimens, with 18% showing a score of "1." Thus, 62% of the specimens revealed minimal or no leakage. Also, 6% and 32% of the specimens showed scores of "2" and "3" (maximum leakage), respectively. The Aegis PP group exhibited: (1) no leakage 90% of the time; and (2) the least number of sealants displaying maximum (scores=2 and 3) leakage at 0%.

The Admira Seal PP group had the fewest sealants without leakage at 10% and the most sealants displaying maximum leakage at 70%.

Penetrability analyses

Sealant penetrability analysis (Table 2) revealed 40 readings per group for 400 total measurements. The Delton PP group revealed the highest (although not necessarily always significant) penetrability. The Aegis PP group revealed lower penetration values than the other groups. Greatest penetration (score=3) was observed in 271 of 400 sections (68%). The remaining penetration values included: (a) score "2" (120 of 400; 30%); and (b) score "1" (9 of 400; 2%). No sections revealed a "0" penetrability.

Fissure type analyses

Fissure type analysis examined microleakage and penetrability of the tested groups. Twenty-nine percent of the specimen sections showed "u" type fissures, with "v," "y^1," and y^2 fissures exhibiting 37%, 25%, and 8%, respectively. Statistical analysis showed no significant differences between fissure types (N=800 compacted), considering specimen group microleakage. Although not significantly different, the mean microleakage scores included: (1) u (228)=0.36; (2) v (300)=0.523; (3) y^1 (202)=0.49; and (4) y^2 (70)=0.60. Significant differences between all fissure types (N=400) were exhibited regarding penetrability. The mean scores included: (1) u (114)=2.92; (2) v (150)=2.81; (3) y^1 (101)=2.38; and (4) y^2 (35)=1.97.

Regression analyses

No There was no correlation was evidenced between sealant microleakage and penetrability of the treatment groups using simple regression analysis (r^2=0.004).

Although significant differences were displayed between treatment groups regarding microleakage and penetrability, considering the interaction of individual impact variables, the material type was the only factor exhibiting a significant role on microleakage. Fissure type was the only independent variable significantly impacting penetrability.

Discussion

Sealant effectiveness is directly related to the enamel surface adaptation and long-term retention of the material. It has been reported that failure rates of sealants can be expected to occur

Table 1. DISTRIBUTION AND STATISTICAL ANALYSIS OF MICROLEAKAGE (N=100/10 GROUPS=10 SPECIMENS/GROUP)*

Group	Microleakage Score			
	0	1	2	3
Aegis PP[a]	9	1	0	0
Aegis AA[ab]	6	3	0	1
Conseal f PP[bc]	4	0	0	6
Conseal f AA[bc]	4	3	0	3
Admira Seal PP[c]	1	2	1	6
Admira Seal AA[bc]	4	1	0	5
Triage PP[c]	1	3	1	5
Triage AA[ab]	5	2	3	0
Delton PP[ab]	5	3	1	1
Delton AA[bc]	5	0	0	5

* Significant differences comparing all groups: $P=.006$ (Kruskal-Wallis test); different superscript letters denote significant differences between paired groups at $P<.05$ (Mann-Whitney test).

AA= air abrasion PP= pumice-prophylaxis

from 5% to 10% per year.[26] Sealant efficacy and clinical success directly correspond to: (1) an understanding of the cariology process; (2) the enamel surface preparation (conditioning technique, material interaction to the appropriate surface); and (3) the type (depth and width) of the fissure involved.[6,8,26]

When these 3 independent parameters can be suitably controlled, effective adhesion of the sealant material to tooth structure can be attained, with ensuing clinical success and longevity.

This study's results revealed that the lowest microleakage scores were associated with Aegis (pumice-prophylaxis and air-abrasion to a lesser degree), compared to the other treatment groups. This result may be related to the degree of material conversion (compatibility with a halogen light source and/or individual material photoinitiator) and material shrinkage, either short-term from the light curing or from long-term polymerization stresses. Shrinkage potentially causes debonding of the resin components from the tooth structure, with resultant leakage of contaminants and possible restoration failure and ensuing replacement.[27]

Additional factors that can potentially contribute to ineffective adaptation and mechanical or chemical surface adhesion include: (1) dehydration of the restorative material following insertion; (2) technique sensitivity during placement; and/or (3) enamel surface contamination. Triage used with either air-abrasion or pumice-prophylaxis surface conditioning showed cracking, possibly due to dehydration following curing. The Triage specimens were coated with a surface sealant following placement and stored (hydrated) in water during the entire experimental protocol to prevent such a dehydration situation from occurring. The Triage groups were among the highest regarding microleakage, possibly due to formation of surface fractures (in spite of application of a surface sealant) causing an ensuing increase in microleakage. These results are in partial agreement with previous studies[28-30] that indicated poor retention rates of glass ionomer-based materials placed as occlusal sealants. A clinical study[31] evaluating glass ionomer-based sealants (Vitremer, 3M-ESPE, St. Paul, Minn, and Ketac Bond, GC America, Alsip, Ill) reported low retention rates. The materials, however, had a cariostatic effect. Alonso et al[32] concluded that Fluoroshield (Dentsply Professional, Mil-

Table 2. DISTRIBUTION AND STATISTICAL ANALYSIS OF SEALANT PENETRABILITY (N=400/10 GROUPS=40 SECTIONS/GROUP)*

Group	Penetration Score			
	0	1	2	3
Aegis PP[c]	0	3 17	20	
Aegis AA[a]	0	1	8	31
Conseal f PP[a]	0	0	9	31
Conseal f AA[ab]	0	3	8	29
Admira Seal PP[abc]	0	0 17	23	
Admira Seal AA[bc]	0	1 18	21	
Triage PP[a]	0	1	7	32
Triage AA[ab]	0	0 14	26	
Delton PP[a]	0	0	7	33
Delton AA[abc]	0	0 15	25	

* Significant difference comparing all groups: $P=.004$ (Kruskal-Wallis test); different superscript letters denote significant differences between paired groups at $P<.05$ (Mann-Whitney test).

AA= air abrasion PP= pumice-prophylaxis

ford, Del) and Vitremer had higher bond strengths to enamel than a conventional glass ionomer cement material.

In this study, microscopic revealed Triage and Aegis surface absorption of the methylene blue dye. Dye absorption into the 2 materials did not necessarily affect microleakage at the material/tooth interface, but could certainly imply long-term staining for an unesthetic result.

Penetrability of the sealants showed significant differences, with Delton and Triage revealing higher (not necessarily always statistically significant) scores than the other groups. It must be noted that Triage manipulation into the occlusal surface grooves and fissures was a bit labor intensive, and the resulting low viscosity and high penetrability was unexpected. Conseal f, marketed as a low viscosity sealant, showed satisfactory, although not the highest, penetrability scores.

This study's results showed no significant differences in surface preparation techniques and, thus, they were not a significant factor regarding sealant microleakage and/or penetrability. These results agree with previous research showing no significant differences between the 2 cleaning techniques.[9,10,33] Although air abrasion may be used for the efficient removal of incipient carious lesions, the present results show that the use of this technique for surface conditioning is too aggressive and, therefore, unnecessary. Additional disadvantages of air abrasion include: (1) indiscriminate release of dust particles in office environment; (2) production of microcracks in the tooth structure; and (3) introduction of fine silicate particles deep into the pits and fissures.[11]

In this study fissure morphology did not exhibit a significant effect on microleakage, although u-shaped fissures showed the least amount of leakage. Significant differences were evident regarding sealant penetrability, however, with u-types and v-types displaying the greatest penetrability, in that order. Y^2-shaped fissures revealed the lowest degree of penetrability. These results agree with previous research[10,20] in which fissure morphology was significantly related to penetrability, but no differences were found for microleakage. One study[22] also reported that sealant penetrability to the base of the fissure occurred more frequently in shallow fissures compared with deep fissures, a fact verified by our results.

No correlation between treatment group microleakage and penetrability was exhibited, as shown by simple regression analysis, which agreed with a previous study.[22] This finding suggests that total penetration of the sealant material is unnecessary if an adequate bond has occurred coronal to the base of the fissure and adjacent to the cuspal inclines. As concluded by Courson et al,[34] "an efficient sealant must have a good sealing ability and a high rate of infiltration as well, but these 2 properties probably do not have the same clinical relevance; furthermore, the imperviousness of the seal remains the most important requirement."

Conclusion

1. Sealant characteristics and fissure morphology were are significant factors regarding sealant success, while surface preparation does not play an important role in sealant microleakage or fissure penetrability.
2. Aegis sealant with either pumice-prophylaxis or air-abrasion surface treatment shows the least microleakage while also showing inferior penetrability characteristics.
3. Delton and Triage (pumice-prophylaxis conditioning) show superior results regarding sealant penetrability.
4. Fissure morphology significantly influences sealant penetrability, though it was is not a factor for microleakage.

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