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Original Article

Dentistry Section

Effect of 30% Grape Seed Extract on the Shearbond Strength of Orthodontic Adhesive Resin: An In-vitro Study

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ABSTRACT

Introduction: Grape Seed Extract (GSE) contains Proanthocyanidins (PA) which have been shown to cross-link and strengthen demineralised dentin collagen. There is paucity of research to evaluate, if grape seed extract increases the Shearbond Strength (SBS) of orthodontic resin when bonding to enamel.

Aim: To evaluate the SBS and Adhesive Remnant Index (ARI) scores of enamel surface bonded with orthodontic resin after immersing in 30% of grape seed extract and compare the same with that of control group.

Materials and Methods: This in-vitro study was conducted in SRM Dental College, Ramapuram, Chennai, India, from January 2021 to August 2021, in which 72 extracted teeth samples were divided into two groups. The two groups, each with 36 specimens, included Group I (Control), Group II (Test). Teeth in the test group were soaked in 30% grape seed extract for 10 minutes before bonding and teeth in the control group were bonded directly without soaking in grape seed extract. SBS were measured using a universal testing machine. To evaluate the amount of resin left on the enamel surfaces after debonding, ARI scores were used. The samples were subsequently evaluated using Scanning Electron Microscope (SEM) to study the surface characteristics of enamel after immersion in grape seed extract. Student's t-test was used to assess the difference between two groups in SBS and Chi-square test was used for ARI scores.

Results: Total of 72 teeth were analysed, 36 extracted teeth in each group. The mean SBS in group I was 78.9 Newton (N), 59.03 N in group II. There was a stastistically significant differences in the mean scores of SBS between the groups (p-value=0.0024). The SBS of control group was significantly higher than the test group. Significant difference (p-value=0.016) found in ARI scores between the two groups. In SEM analysis, after grape seed extract conditioning followed by acid etching, pronounced cobble stone appearance was noticed, indicating a type II etching pattern.

Conclusion: Adhesive failure occurred at the resin and bracket interface indicating that SBS reduces, when teeth were immersed in grape seed extract.

Keywords: Adhesive remnant index, Antioxidants, Enamel conditioning, Proanthocyanidin

INTRODUCTION

Orthodontic mechanotherapy involves application of optimum force to achieve movement of teeth. Traditionally, this force is transmitted to the teeth by brackets bonded to them. This attachment necessitates a dimensionally stable bonding medium that can achieve adequate flow to penetrate the conditioned enamel surface and has exceptional bond strength. It is vital to carefully prepare the enamel surface, in order to get a good and stable bond [1].

To improve the dentin/resin interface properties, two key techniques were considered: the first was to improve and develop novel adhesive systems and the other method was to establish tissue engineering approach to improve the substrate's intrinsic properties. [2]. Srinivasulu S et al., concluded that proanthocyanidin, have been shown to crosslink collagen agent and increase the mechanical characteristics of collagen and its resistance to enzymatic degradation [3]. Phenol is abundant in grapes, particularly in the skin and seeds. Grape seed extract contains a number of bioactive properties, but it is hypothesised that its high concentration of proanthocyanidins (PACs) contributes to its caries prevention ability. Grape seed extracts capacity to bind to proteins may also help with dental remineralisation [4]. In restorative dentistry, grape seed extract has been shown to improve the bonding of composite to dentin [5].

Several factors influence SBS of resin used for bonding brackets to enamel during orthodontic treatment, which can be divided into enamel factors (fillings, hyperplastic or hypoplastic enamel), patient factors (biting on hard surfaces, unpleasant oral habits, poor oral cleanliness) and placement procedures used [2]. There is a paucity of research on the effect of preconditioning agents like GSE on SBS of orthodontic resins when bonding brackets to enamel. Therefore, the goal of the present research was to explore, if conditioning enamel using GSE before bonding improves the SBS of orthodontic resin at bracket to enamel interface.

Study objectives:

- To evaluate SBS of orthodontic resin bonded to enamel preconditioned with immersion in 30% and to compare the same with control.
- To evaluate of Adhesive Remnant Index (ARI) score on enamel surface after debonding of brackets from specimens subjected to enamel preconditioning and compare with control.
- To assess the enamel and dentinal surface on teeth subjected to preconditioning with GSE and compare that with the control.
- The study considered null hypothesis as there is no difference in the SBS and ARI score between the GSE treated group and control group.

MATERIALS AND METHODS

This in-vitro study was conducted in SRM Dental College, Ramapuram, Chennai, India, from January 2021 to August 2021. The study protocol was approved by the Institutional Review Board of SRM Dental college, Ramapuram, Chennai (SRMDC/IRB/2019/MDS/105).

Sample size calculation: It was done using G power software version 3.1.9.7 and for a power of 80 and α error of 0.05, total sample size arrived at was 72 [6].

Inclusion criteria: Extracted premolar teeth collected after extraction from patients who required therapeutic extraction of premolars for orthodontic reasons were included in the study.

Exclusion criteria: Teeth with dental filling or loss of tooth structure on buccal side of tooth (the surface to be bonded with bracket), teeth with large cavity or decay/dental caries on the tooth surface, teeth with crack that can affect the strength of enamel, teeth that were pretreated with chemical agents and tooth morphologic abnormalities like hypocalcified, hyperfluorosis etc., were excluded from the study.

Study Procedure

The teeth were extracted and stored for one week in a 0.5% chloramine T solution for the purpose of disinfection before being transferred to distilled water and kept at room temperature until the experiment. The teeth were then mounted in acrylic blocks so that long axis of the tooth coincided with that of the acrylic. Debris or calculus on the tooth surface was removed by scaling, which was then polished for 15 seconds with non fluoridated pumice and rubber prophylactic cups, rinsed with water spray for 10 seconds, and dried with oil-free compressed air for 10 seconds.

Thirty-six extracted premolar teeth were used as control (Group1) and were labelled as (C1-C36). According to the manufacturer's instructions, the buccal surfaces were etched with a 37% phosphoric acid solution for 30 seconds rinsed with water spray for 20 seconds, and then left to dry with oil-free compressed air for 20 seconds. Trans bond XT Primer (3M Unitek Pvt. Ltd.) was applied on the etched enamel and cured for 20 seconds and 3M Universal Gemini upper premolar brackets were bonded on the middle third of the enamel parallel to the long axis with the composite resin (Transbond XT 3M Unitek) and cured for 40 seconds.

The 30% GSE was made using the Soxhlet extraction process [6]. Rest of the collected (36) premolar teeth were used as test (Group 2) and was labelled as (T1-T36). Teeth were soaked in GSE for 10 minutes [6]. In the main chamber of the Soxhlet extractor, 40 g of grape seeds (Herabal Engine) were placed in a thimble (thick filter paper bag). In a distillation flask, the Soxhlet extractor was inserted with 40 mL N-hexane as the extraction solvent. The solvent was then heated to reflux temperature. The container was gradually filled with warm solvent while the solid substance was heated. The Soxhlet chamber was automatically emptied, when it was nearly full, with the solvent running back down to the distillation flask through a syphon side arm. Over the period of five hours, this cycle was repeated numerous times. During each cycle, a component of the non volatile chemical was dissolved in a solution. After several cycles, the needed component was concentrated in the distillation flask. After extraction, the solvent was removed using a rotary evaporator. 30 mL of this extract was diluted in 70 mL ethanol to make a 30% GSE solution and preserved in a tightly sealed bottle [6].

Thirty-six teeth of the test group were soaked in 30% GSE for ten minutes before etching and rinsed in running water for five seconds [5]. After cleaning, teeth were washed and dried using oil-free compressed air. The buccal surfaces then were etched with a 37% phosphoric acid solution for 30 seconds, washed with water spray for 20 seconds, and dried with oil-free compressed air for 20 seconds, as directed by the manufacturer. Transbond XT Primer was applied on the etched enamel and cured for 20 seconds and 3M Universal Gemini upper premolar brackets placed on the middle-third of the enamel parallel to the long axis with the composite resin (Transbond XT) and cured for 40 seconds. Excess composite was removed from around the bracket margins with the tip of a probe, and each of the five directions was photopolymerised for 20 seconds: above the bracket, occlusal, cervical, mesial, and distal surfaces.

Debonding force was tested using an Instron Universal Testing Machine with a cross head speed of 0.5 mm/min and 50 kg load cell. The acrylic blocks were attached onto the Instron testing machine's attachment, sample was secured tight and brackets were delivered with a shear force to debond them with an upper crosshead blade that glides upwards or downwards with a configurable speed. The debonding force at failure was recorded in Newton (N). The SBS, in Megapascal (MPa), was then calculated as follows: [7]

- The SBS was calculated using the formula, SBS=Force/surface Area of the bracket base
- The surface area of the bracket base is depicted as A. the surface area of Gemini series (3M Unitek, Monrovia, Calif.), MBT prescription 0.022 slot, upper premolar brackets is 10.5 mm^2.
- F is the recorded force [by the Instron machine].

After debonding the bracket, the enamel surface was evaluated based on the modified ARI score to determine the type of fracture, whether it was an adhesive or cohesive fracture.

The following criteria were used to assign the scores: [8,9]

Score 0=No adhesive left on the tooth surface

Score 1=1-25% of the adhesive left on the tooth surface

Score 2=26-50% of the adhesive left on the tooth surface

Score 3=51-75% of the adhesive left on the tooth surface

Score 4=76-99% of the adhesive left on the tooth surface

Score 5=100% of the adhesive left on the tooth surface

Two teeth were randomly selected for SEM study, one of them was used as a control and while the other tooth was soaked with GSE for 10 minutes. Teeth were sectioned with rotary handpiece, cut being 3 mm lingual to the tip of the buccal cusp to ensure that buccal surface enamel was untouched and free for conditioning along with exposed dentin. Teeth were sectioned before soaking in GSE so that dentinal tubules can also be studied. After sectioning, the test tooth was soaked in 30% GSE followed by acid etching for 30 seconds of both specimens. The samples were then kept isolated and dried for 24 hours to remove all moisture, which could interfere with the vacuum needed for metallisation. All samples were then conventionally metallised (Gold sputtering JEOL JFC 1100E) and observed under SEM. The SEM device works on principle of conducting current through the sample of interest, as tooth is a biological entity, it had to be coated with the conducting agent to prevent burn out of the sample; hence, gold sputtering was carried out. Samples were examined under different magnification to show surface properties at 200X and 1000X.

STATISTICAL ANALYSIS

Data was assessed for normality using Shapiro-Wilk test. Descriptive statistics for the SBS, including the mean, standard deviation and minimum and maximum values were calculated for each of the two groups of teeth tested for SBS. Since the data was normally distributed Student's t-test was used to determine the differences in SBS existed between the groups. Since ARI Scores were obtained as categorical data Chi-square test was done to compare the ARI scores.

RESULTS

The mean debonding force in group I (control) was 78.9 N, 59.03 N in group II (Test) [Table/Fig-1]. The mean SBS in test group was 5.62 Mpa and 7.51 Mpa in control group [Table/Fig-2]. There was a statistically significant difference in the mean scores of SBS between the groups (p-value=0.024). Null hypothesis was, therefore, rejected. The SBS of control group was significantly higher than the test group. This indicates that immersing in GSE reduces the SBS of composite resin used on enamel.

Comparison of qualitative ARI scores between the groups was done using Chi-square test [Table/Fig-3]. None of the samples in the test group had score of 1 or 2 (n=0) whereas in the control group there were few samples showing score 1 and 2 (n=5 for 1

Group	Mean	Std. Deviation	Std. Error Mean	p-value
Control	78.9032 (N)	41.65281	7.48107	0.0024*
Test	59.0323 (N)	23.28874	4.18278	

[Table/Fig-1]: Comparison of SBS between the groups in Newtons (N). Student's t-test, *p<0.05 indicates significant

Group	Mean	Std. Deviation	Std. Error Mean	p-value
Control	7.514593 (Mpa)	3.9669348	0.7124825	0.0024*
Test	5.622120 (Mpa)	2.2179756	0.3983602	

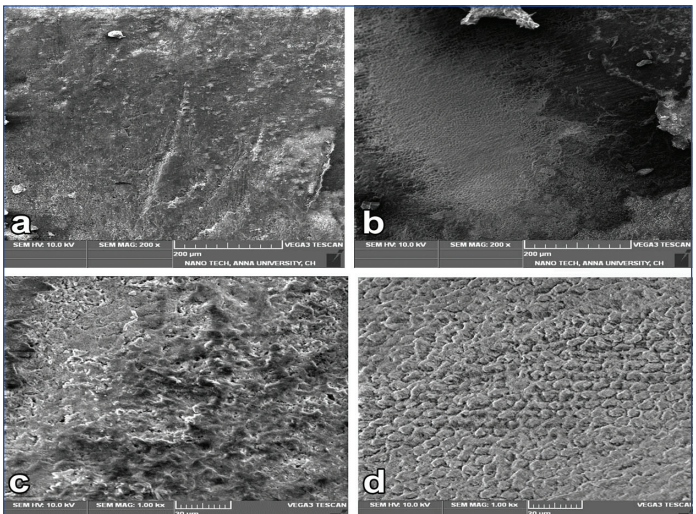
[Table/Fig-2]: Comparison of SBS between the groups in Megapascal (Mpa). Student's t-test, *p<0.05 indicates significant

and n=3 for score 2) in the control group. Number of samples with score 4 were 8 (22.2%) in control group and 5 (13.9%) in test group. When samples in score 5 were compared, both the groups had maximum number of samples (n=23 for test group and 12 for control group). Though both the groups had maximum number of samples in score 5, it was statistically high for test group (p=0.016) which indicates adhesive was left behind on the tooth in increased number of samples in test group than control.

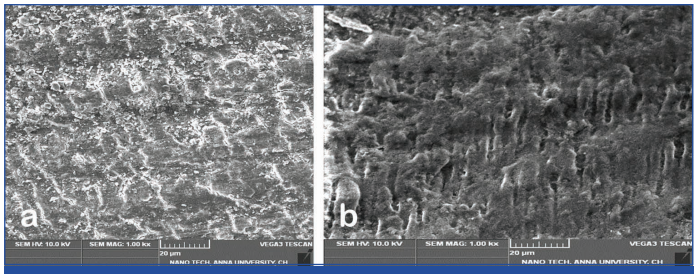
Adhesive Remnant Index score	% of cases	Group		Chi-square statistic	p-value
		Control	Test		
1-25% left	Count	5	0	12.149	0.016
	% within group	13.9%	0.0%		
26-50% left	Count	3	0		
	% within group	8.3%	0.0%		
51-75% left	Count	8	8		
	% within group	22.2%	22.2%		
76-99% left	Count	8	5		
	% within group	22.2%	13.9%		
100% left	Count	12	23		
	% within group	33.3%	63.9.0%		

[Table/Fig-3]: Comparison of Adhesive Remnant Index (ARI) Scores between control and test groups.

In the present study, SEM analysis of enamel etched with phosphoric acid showed ill-defined prisms in both 200x [Table/Fig-4(a)] and 1000x magnification [Table/Fig-4a]. In contrast, cobble stone appearance (type II etching pattern) of enamel was noticed when the sample was conditioned with GSE before etching at 200X [Table/Fig-4(c)] and 1000X [Table/Fig-4(d)] [10]. When studying dentin using SEM, it was found that the test tooth immersed in GSE showed well-defined dentinal tubules when, compared to the control tooth [Table/Fig-5a,5b].



[Table/Fig-4]: Enamel surface without conditioning with grape seed extract at a) 200X and b) 1000X magnification showing ill-defined prismatic structure. After conditioning showing pronounced cobble stone pattern c) 200X and d) 1000X magnification.



[Table/Fig-5]: Dentinal surface without conditioning with grape seed extract at 1000X magnification showing ill-defined prismatic structure (a). After conditioning showing well-defined tubules (b).

DISCUSSION

Many researchers have studied the importance of enamel surface preparation prior to the attachment of orthodontic brackets, as well as SBS during bracket debonding [11,12]. Grape seed is a phenolic substance with the potential to cross-link collagen, which helps to maintain the durability of dentin collagen matrix and thus improving biodegradation resistance, but its effect on enamel has not yet been investigated. Proanthocyanidin could be included in an orthodontic adhesive system, as it can increase the collagen cross linkage [5]. Xie Q et al., used 6.5 wt % of GSE to study its remineralising capacity in artificial root caries and detected a positive effect on remineralisation [4]. Green B et al., studied the morphological difference in hybrid layer created by Bisphenol A-glycidyl Methacrylate (Bis-GMA)/ 2-Hydroxylethyl Methacrylate (HEMA) with and without 5% GSE and found out that collagen fibril degradation was prevented by the antioxidants in grape seed extract [2]. Mirkarimi M et al., performed an experiment of human primary molars using GSE and found out that there was an increase in microhardness of enamel in 12 wt% grape extract group and there was an enhanced remineralisation of artificially created lesions [13]. A study by Generosa DM et al., proved that 2.9 wt% of GSE improved the SBS of composite resin bonded to dentin [5] Shahi M et al., compared the effect of guava seed extract solutions in various concentrations (10%, 20%, 30%) on the SBS of composite resin to bleached enamel and concluded that Guava seed extract showed a complete reversal of the compromised bond strength with increased concentration [6]. In the present study, concentration of 30% was arrived to extract the maximum benefits of antioxidants present in grape seed extract. Shahi M et al., investigated the efficacy of guava seed extract in concentrations of 10, 20 and 30% in reversing the bonding abilities of composite resin on bleached enamel [6]. They found out that samples immersed in 30% of the extract yielded highest SBS regardless of the time of immersion. As both grape seed and guava seed belong to the same group of PA, the same concentration of 30% was tested using GSE in this study [2,6] .

Reynolds IR and Von Fraunhofer JA suggested the clinically acceptable bond strength of brackets to enamel to be 6-8 MPa [14]. The SBS of control group was significantly higher than the test group. This indicates that preconditioning with GSE reduces the SBS of orthodontic resins bonded to enamel. This observation is in contradiction to various studies that have tested the effect of preconditioning agents like antioxidants on the SBS of restorative adhesive resins bonded to dentin [2-5]. Generosa DM et al., reported highest mean value of the SBS with 2.9% GSE before etching the dentinal surface for 10 minutes to be 3.34 Mpa and 6.9 Mpa in control group [5]. Subramonian R et al., compared the shearbond of composite resin bonded to bleached enamel after pre conditioning with 10% pine bark extract application that yielded the highest SBS among the test groups (10.8±1.25 Mpa) [15]. [Table/Fig-6] depicts the comparative analysis of concentration of GSE and its effects observed in past studies with that of present study [2-5,13,15].

Hybrid layer is a resin, collagen, and dentin intermediate layer created by acid etching the dentin and resin infiltration into the conditioned dentin. Adhesive monomers are unable to fully encapsulate the

S. No.	Authors name and year	Place of study	Sample size	GSE concentration used	Parameters compared	Conclusion
1	Xie Q et al., (2008) [4]	Chicago	Twenty five extracted human third molars	6.5%	Remineralisation of artificial root caries	GSE positively affects remineralisation process of artificial root caries
2	Green B et al., (2010) [2]	USA	Eight extracted human molars	5.0 wt%	Morphological differences of hybrid layers created by BisGMA/HEMA with and without GSE	Presence of GSE in dental adhesives inhibited the biodegradation of unprotected collagen fibrils within the hybrid layer
3	Mirkarimi M et al., (2013)[13]	Iran	Seventeen human sound primary incisors	90%	Microhardness and remineralisation of artificial enamel lesions	GSE enhanced microhardness and remineralisation of artificial enamel lesions
4	Subramonian R et al., (2015) [15]	India	Fifteen human premolars in each group	10%	Effect of 10% sodium ascorbate,10% of GSE, 10% of pine bark extract on the SBS to bleached and unbleached enamel	Unbleached teeth showed highest SBS followed by bleached teeth treated with 10% pine bark extract
5	Generosa DM et al., (2017) [5]	Indonesia	Twenty four extracted teeth	2.9%	Effect of GSE on resin dentin SBS	Highest SBS in the group treated with GSE before etching
6	Present study	India	Seventy two extracted teeth	30%	Effect of GSE on resin enamel SBS.	Decreased SBS in the group treated with GSE before etching but increase in ARI scores and well-defined prisms in SEM analysis

[Table/Fig-6]: Similar studies from the literature [2-5,13,15].
USA: United states of America; BisGMA: Bisphenol a-glycidyl methacrylate; HEMA: 2-Hydroxylethyl methacrylate; GSE: Grape seed extract

collagen matrix, leaving behind exposed collagen fibrils at the bottom of the hybrid layer, which are not protected by polymerised resin which leads to increase in susceptibility of demineralised collagen fibrils, and they become susceptible to hydrolytic breakdown over time [16]. Tooth restoration methods causes activation of Matrix Metalloproteinase (MMP) enzyme by the total etch technique, which reduces the resin bond strength and GSE has been shown to improve this bond strength in various studies [2-5]. It is evident from this study that, the same is not applicable for enamel.

Proanthocyanidin (PA), a powerful antioxidant cross-linking agent found in fruits, vegetables, nuts, seeds, and flowers, has a wide range of biological actions. The use of a grape seed extract, which is mostly made up of PA, has been demonstrated to improve the mechanical qualities of dentin by improving collagen cross-linking and thereby, resisting biodegradation [2].

Proanthocyanidins have both hydrophobic and hydrophilic properties, enhancing their ability to irreversibly connect to a number of substances, including minerals, proteins, and carbohydrates. GSE's binding to carbohydrate substrates required for bacterial development may reduce biofilm formation on the tooth surface. GSE's capacity to bind to proteins may also help with dental remineralisation [4]. Prior to bonding treatments on bleached enamel, the application of GSE entirely neutralises the bleaching effects and considerably improves bond strength [15,17].

A modified ARI score index as given by Cehreli was utilised to grade the proportion of residual adhesive left behind on the enamel after the SBS test [8]. In the current study, there was a significant difference in ARI scores between the two groups. A total of 77.7% of control group participants was spread across all the scores with increased incidence of scores 1-3. The results of ARI scores indicate that adhesive failure between bracket base and resin surface was the reason for bond failure in the test group. It is evident from the study, that GSE definitely has an effect on reducing the bond strength of orthodontic resin on enamel and the mechanism is by adhesive failure between resin and bracket interface rather than resin enamel interface. Role of the antioxidants in GSE in causing adhesive failure between bracket and resin is still unclear and has to be studied further. A similar study by Bulut H et al., compared SBS of bleached enamel against unbleached enamel with and without antioxidant application. They found that unbleached enamel had the highest bond strength with high ARI scores followed by bleached enamel immersed in artificial saliva and antioxidant [17].

In the present study, SEM analysis of enamel etched with phosphoric acid showed ill-defined prisms in 1000X magnification. But after GSE conditioning followed by acid etching, pronounced cobble stone appearance was noticed indicating a type II etching pattern [10]. The dentinal tubules also showed well-defined tubules when preconditioned with GSE more than the conventional group, which indicated that GSE had better ability to remove the smear layer [5]. similar studies have been tabulated in [Table/Fig-6] [2,4,5,13,15].

In the current study, there was reduction in SBS of orthodontic brackets bonded to enamel after preconditioning with grape seed extract. This indicates that the effect of GSE on the bond strength of adhesives is different, when treated on enamel and dentin. Hence, the use of GSE as a preconditioning agent to increase the SBS in orthodontic bonding is questionable.

Orthodontists are concerned with the bond strength of the attachments because they have to be intentionally removed upon completion of treatment. Excessive bond strength has the potential to harm enamel surfaces. An appropriate bond strength range should be high enough to prevent bracket debonding issues. With the findings of the present study, the previously stated null hypothesis was rejected. Hence, the conditioning of 30% GSE on enamel surface for a duration of 10 minutes show significant decrease in SBS, though ARI scores and SEM images show favourable results.

Limitation(s)

Preconditioning of enamel was done using GSE in the present study and other reagents like guava seed extract, green tea extract etc., could have been used for comparative purpose.

CONCLUSION(S)

Immersion of teeth in GSE before bonding reduces the SBS of orthodontic resins bonded to enamel surface. ARI scores were were significantly lower in treated enamel, thus adhesive failure occurred at the resin and bracket interface and not at enamel. SEM images indicated a perfect cobble stone appearance of etched enamel surface in the test tooth. There is a need for further research in the same topic to understand the bonding behaviour of resins on enamel surface preconditioned with reagents. Further research needed to assess the effectiveness of proanthocyanidin by incorporating it into adhesives in different concentration. It is an in-vitro study, further research using the same reagent can be done on enamel that, is affected by fluorosis and hypoplastic tooth to determine whether there is any effect on orthodontic bonding. Other concentrations like 10, 20% can be tested, to find out if, there is reversal of results.

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