MICRO SHEAR BOND STRENGTH ASSESSMENT OF TWO DIFFERENT ADHESIVE SYSTEMS BONDED TO DIFFERENT DENTIN SUBSTRATES

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ABSTRACT

This in-vitro study assessed micro shear bond strength of two different adhesive systems bonded on different dentin substrates. Fifteen freshly extracted human molars with coronal dentin caries were collected for this study. The roots were sectioned to expose the pulp chamber. The crown segment was then centrally glued using a cyanoacrylate adhesive to a circular Teflon plate of 10 mm diameter and 2 mm thickness. The occlusal portion of crown segments were ground flat to expose flat dentin surface parallel to the occlusal plan. Caries detector dye was used to identify caries-infected, caries-affected (CAD) and normal dentin (ND). For further confirmation, dye permeability test was applied to detect CAD. Specimens were divided into two main groups (30 each) according to the type of the adhesive system used: A1 (Adper Scotchbond Multipurpose adhesive) control group and A2 (Universal adhesive system). Each group was subdivided into two subgroups (15 specimens each) according to the type of dentin substrate used: D1 (ND) and D2 (CAD). Four resin composite microcylinders were built over each specimen in both substrates. A total of 60 composite microcylinders with prepared dentin surface containing both ND and CAD were used in the study. Each was polymerized using light curing unit. All specimens were subjected to micro-shear bond strength (μSBS) testing using a special universal testing machine (Model LRX-plus; Lloyd instruments Ltd., Ferham, UK). Data was then recorded, tabulated and statistically analyzed. Single bond Universal adhesive system (20.0 ± 96.7 MPa) showed statistically significantly lower mean μSBS than Adper Scotchbond Multipurpose adhesive systems (22.0 ± 10.4 MPa) (P=0.008). Micro-shear bond strength (μSBS) of ND substrate (24.3 ± 8.4 MPa) showed higher statistically significant values than that of CAD (19.4 ± 7.8 MPa) (P=0.001*). Two way ANOVA results showed statistical significant effect for the adhesive systems (Adper Scotchbond Multipurpose and Single bond Universal Adhesive) [p=0.008], dentin substrates (ND and CAD) [p<0.001] and the interaction between the variables had a statistically significant effect on the mean micro-shear bond strength. In conclusion, the type of substrate and bonding strategies affects the bonding performance and durability of restorations in dentistry.

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INTRODUCTION

There are many factors that influence the durability of the resin composite restorations; the most important is the interfacial integrity. Over the past decades, many attempts of making alterations to the adhesive systems and techniques have been exerted by scientists in order to attain satisfactory hybridization of enamel and dentin, and formation of hybrid layer, which have resulted in improved bond strength (**Müller et al, 2017**).

Due to material handling problems, many studies have focused on simplified versions of dentin bonding systems. Recently, simplified 'all-in-one' adhesive systems, also referred to as one-step self-etch adhesive system, have become popular for functional and aesthetic reconstruction of debilitated tooth structure, together with conservation of dental tissues, since they are particularly simple and rapid at application. However other researches has shown that such a system cannot achieve the same outcome as a three step etch-and-rinse bonding system for achieving efficient and stable bonding to both enamel and dentin (**Tuncer et al, in 2013, Makishi et al, in 2011, Van Meerbeek et al, in 2010,** and **Frankenberger et al, in 2001**).

The most dominant studies in conservative dentistry are thus far related to the bonding to normal dentin. Major efforts were exerted for the differentiation between infected dentin and caries-affected dentin which has the capability of remineralization throughout the application of different materials which is used for selective caries removal techniques so as to prevent exposure of the pulp. These have resulted in a cavity floor formed of caries-affected dentin after the removal of the infected dentin, in which resin composite restoration can be perfectly bonded to this prepared cavity (**Müller et al, 2017**).

The current dentin adhesives exhibit high bond strength values to the tooth structure. However, dentin bonding depends not only on the adhesive system but also on the dentin substrate. Clinically, cari-

ous dentin has two layers: the outer one consists of bacterial infected dentin, while the inner layer is the caries-affected dentin and has the capability of remineralization, since most bonding occurs on caries affected dentin, this may affect long-lasting bonding of composite restorations, as caries-affected dentin contains tubules that are filled with acid-resistant minerals produced by demineralization-remineralization cycles which interfere with the infiltration of adhesive systems and prevent resin tag formation during bonding During laboratory assessment, the efficacy of the adhesive systems in both normal and caries-affected dentin should be taken into consideration given that, clinically, bonding takes place in the combination of both (**Shibata et al, in 2016, de Almeida Neves et al, in 2011,** and **Wang et al, in 2007**).

Thus, the aim of the current in-vitro study was to assess micro shear bond strength of two different adhesive systems bonded on different dentin substrates.

MATERIALS AND METHODS

Table 1 showed the different materials used in this study including the composition and manufacturer of each material, and they are:

- Three-step etch-and-rinse adhesive system: Adper Scotchbond Multipurpose,

- One-step self-etch universal adhesive system: Single bond Universal Adhesive system,

- Composite resin restoration: Filtek Z350 xt, shade A2,

- Caries detector dye: Seek, is used to differentiate between caries-affected dentin (CAD) and normal dentin (ND).

- Methylene blue dye: is used to differentiate between CAD and ND by dye permeability test. It was prepared by dissolving 100 gm of the dye powder in one liter of distilled water to obtain 10% concentration of methylene blue.

A total of fifteen freshly extracted human molars with coronal dentin caries were collected for this study. The carious teeth included in this study were selected according to the following criteria:

- Carious lesion involving only the occlusal surface.

- The carious lesion was not extending in width more than two thirds the occlusal surfaces to be able to attain ND at the same level with CAD.

- The type of the carious lesion was chronic decay. On visual examination, it showed to be with dark brown or black discoloration. With tactile examination using an explorer, the carious lesion showed relative hard consistency.

Two lines were drawn in all teeth using a permanent marker. The first line denotes the cemento-enamel junction (CEJ) and the second one was 2 mm below the cemento-enamel junction. Afterward, the sectioning of the roots was done at the level of the second line in order to expose the pulp chamber by the aid of double sided diamond disc. The pulpal tissue was carefully removed with a discoid excavator without touching the walls of the pulp chamber, followed by irrigation using saline solution.

The crown segment was then centrally glued using a cyanoacrylate adhesive to circular Teflon plate of 10 mm diameter and 2 mm thickness which had a central hole of 0.5 mm diameter. Size 19 gauge stainless steel butterfly needle was checked to be fit to the central hole of the Teflon plate and to the pulp chamber of the tooth. Then, the needle was detached from the plate and the crown segment.

A plastic rounded shaped mold of (2 cm diameter, 2 cm height) was used as mold for embedding the crown segments. The mold was fitted around the crown segment attached to the plate while the butterfly needle penetrates through the plate to the pulp chamber. A chemically cured acrylic resin was mixed and flowed around the needle, the plate,

and the crown segment to the level of the CEJ. After setting of the acrylic resin, the needle and the embedded specimen were removed from the mold.

The occlusal portion of crown segments were ground flat using double sided diamond disc mounted to low speed hand-piece running under copious water coolant to expose flat dentin surface parallel to the occlusal plan. After flattening of dentin surface, two different methods were used to identify caries-infected, CAD and ND. The first method involved the use of caries detector dye in which the entire flat dentin surface was flooded with caries detector dye for 10 seconds, rinsed for five seconds and then air dried thoroughly. After drying, three different colors were revealed; dark blue denoting caries-infected dentin, light blue color denoting CAD and yellow color considered as ND. Partial removal of dark blue stained caries-infected dentin was done using sharp spoon excavator.

For further confirmation, another method; dye permeability test was employed to detect CAD. In this test, a 19 gauge stainless steel butterfly needle was centrally fitted to the Teflon plate which was glued to the prepared crown segment. At the same time, the other end of butterfly needle was connected to a plastic syringe filled with 10% methylene blue dye so that the dye permeated into the tooth through the pulp chamber under pressure. The ND was stained blue while the remaining carious lesion remained unstained.

To ensure flattening of the dentin surface after CAD identification, a round bur was used and a central indentation was made in the dentin till the head of the bur disappeared. Then, the occlusal surface was flattened. This ensured to have the CAD and ND in one flat plane perpendicular to the long axis of the tooth with no pulpal involvement.

A total of 60 composite microcylinders with prepared dentin surface containing both ND and CAD were used in the study. Specimens were divided into

two main groups (30 each) according to the type of the adhesive system used: A1 (Adper Scotchbond Multipurpose adhesive) control group, and A2 (Universal adhesive system). Each group was subdivided into two subgroups (15 specimens each) according to the type of dentin substrate used: D1 (ND) and D2 (CAD).

-   Adper Scotchbond Multipurpose adhesive bonded to ND group (A1D1)

-   Adper Scotchbond Multipurpose adhesive bonded to CAD group (A1D2)

-   Single bond universal adhesive bonded to ND group (A2D1)

-   Single bond universal adhesive bonded to CAD group (A2D2)

Each one of the adhesive systems used in the study was applied on the entire surface of the dentin specimens according to the manufacturers' instructions:

-   Adper Scotchbond Multipurpose adhesive: Acid etching was performed using 37% phosphoric acid which was applied on the dentin surface for 15 seconds, rinsed with water for 15 seconds and air-dried for 10 seconds leaving dentin moist. Then, primer was applied and air-stream was applied for 10 seconds. Adhesive was applied and gently air thinned for three seconds. Then, adhesive was light cured for 20 seconds.

-   Single bond universal adhesive (self etch mode): The adhesive was applied on the dentin surface using disposable applicator and rubbed for 20 seconds. Then, a direct gentle steam of air was applied for about five seconds until the adhesive film no longer moves. Like other adhesives, light cure was applied for 20 seconds.

A transparent polyethylene tube obtained from a scalp vein infusion set with external diameter of 1.1mm and internal diameter of 0.9 mm was used to aid in resin composite packing. The transparent polyethylene tube was cut into small irises of 0.2 mm length using a No.11 sharp lancet.

Each resin composite microcylinder was polymerized for 20 seconds using Elipar FreeLight 2 LED Curing Light (3M ESPE, Minnesota, USA). The polyethylene irises were then cautiously removed after 24 hours by the aid of the lancet, leaving the composite microcylinders bonded to dentin surfaces. A total of four resin composite microcylinders were built over each specimen in which two microcylinders were built on ND and two on CAD.

All specimens were subjected to micro-shear bond strength (μSBS) testing using a special universal testing machine (Model LRX-plus; Lloyd instruments Ltd., Ferham, UK). The calculation of micro-shear bond strength was done by dividing the load at failure by the bonding area to express the bond strength in MPa according to the following equation: $S = P / \pi r^2$ where: S= Shear bond strength (in MPa); P= Load at failure (in Newton); π= 3.14; r= radius of composite microcylinder (in mm).

Data were collected, tabulated and subjected to statistical analysis. Data were presented as mean and standard deviation (SD) values. Data were explored for normality by checking the data distribution, calculating the mean and median values and using Kolmogorov-Smirnov and Shapiro-Wilk tests. All data showed parametric distribution, so regression model using two-way Analysis of Variance (ANOVA) was used in testing significance for the effect of adhesive system, dentin substrate, and their interactions on mean micro-shear bond strength. Bonferroni's post-hoc test was used for pair-wise comparison between the groups when ANOVA test is significant. The significance level was set at P ≤ 0.05. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

TABLE (1) Material specifications, manufacturers and compositions

Material	Manufacturer	Descriptions/ Compositions
Filtek Z350 xt	3M ESPE, St. Paul – MN, USA	Bis-GMA, UDMA, TEGDMA, and bis-EMA. Fillers: 20 nm silica and 4 to 11 nm zirconia particles, the inorganic filler 78.5% by weight (63.3% by volume)
Single Bond Universal	3M ESPE, St. Paul – MN, USA	A one-step universal adhesive system MDP phosphate monomer, dimethacrylate resins, HEMA, methacrylate-modified polyalkenoic acid copolymer, filler, ethanol, water, initiators, silane,
Adper Scotchbond Multipurpose	3M ESPE, St. Paul – MN, USA	Three-step, etch-and-rinse adhesive system. Etchant:37% phosphoric acid (pH < 1). Primer: Ethanol, HEMA and polyalkenoic acid. Adhesive: HEMA and Bis-GMA.
Seek Caries detector dye	Ultradent Products, South Jordan, UT, USA	Cosmetic red dyes dissolved in propylene glycol base.
Methylene blue dye	Sigma Aldrich, St. Louis, MO, USA	96% methylene blue, 0.5% sulphate ash, 0.005% zinc.

RESULTS

This study was carried out to assess the micro-shear bond strength of etch-and-rinse adhesive system and Universal adhesive system bonded to ND and CAD.

Table (2) and Figure (1) show mean and standard deviation of the micro-shear bond strength (μSBS) values (in MPa) of the two adhesive systems as independent variable. Single bond Universal adhesive system (20.0±96.7MPa) showed statistically significantly lower mean μSBS than Adper Scotchbond Multipurpose adhesive systems (22.0 ± 10.4 MPa) (P=0.008).
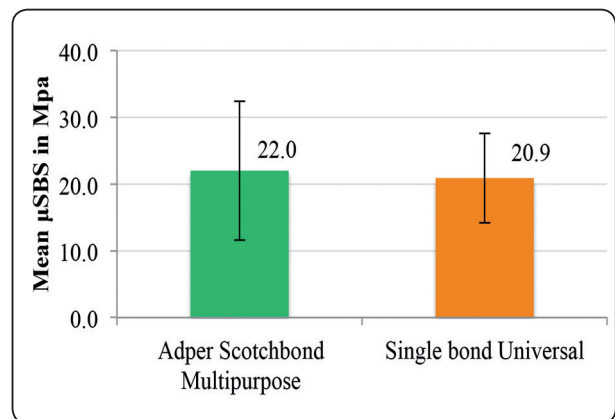

Fig. (1): Bar chart representing mean and SD values of the μSBS of the two adhesive systems as independent variable

TABLE (2): Mean, SD and statistical significance of the μSBS values (in MPa) of the two adhesive systems as independent variable

Adper Scotchbond Multipurpose (Etch-and-rinse)		Single bond Universal (universal self-etch)		P-value
Mean	SD	Mean	SD	
22.0 [a]	10.4	20.9 [b]	6.7	0.008*

: Significant at P ≤ 0.05, Different superscripts in the same row are statistically significantly different

Table (3) and Figure (2) shows mean and standard deviation the micro-shear bond strength values of the two dentin substrates. Micro-shear bond strength (μSBS) of ND substrate (24.3±8.4 MPa) showed higher statistically significant values than that of CAD (19.4 ± 7.8 MPa) (P=0.001*).

TABLE (3): Mean, standard deviation and statistical significance of the μSBS values in (MPa) of the two dentin substrates

Normal dentin		Caries-affected dentin		P-value
Mean	SD	Mean	SD	
24.3 [a]	8.4	19.4 [b]	7.8	<0.001*

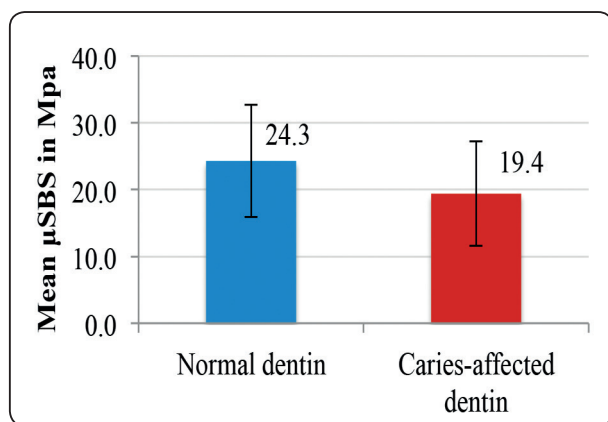*: Significant at P ≤ 0.05, Different superscripts in the same row are statistically significantly different*

Fig. (2): Bar chart representing mean and SD values of the μSBS of the two dentin substrates

Two way ANOVA results showed statistical significant effect for the adhesive systems (Adper Scotchbond Multipurpose and Single bond Universal Adhesive) [p=0.008], dentin substrates (ND and CAD) [p<0.001] and the interaction between the variables had a statistically significant effect on the mean micro-shear bond strength.

Table (5) shows the comparison of the effect of different adhesive systems and dentin substrates on the mean micro-shear bond strength by two-way ANOVA. Since the interaction between the variables is significant, then the variables are dependent upon each other.

Table (4): Two-way ANOVA testing of the study variables

Source of Variation	Type III Sum of Squares	Df	Mean Square	F-value	P-value
Adhesive system	145.2	2	72.6	4.9	0.008*
Dentin substrate	1421.3	1	1421.3	96.3	<0.001*
Adhesive system x Dentin Substrate	144.6	2	72.3	4.9	0.008*

*df: degrees of freedom = (n-1), *: Significant at P ≤ 0.05*

DISCUSSION

Nowadays, the recent innovations of bonding materials has lead to the evolution of the science of adhesive dentistry. Many researches have been conducted on the different types of adhesive systems. The universal or multimode adhesive systems are lately introduced, and they are characterized by their simplicity as they are contained in one bottle. They may be applied using etch and rinse mode or self etching mode **(Isolan et al, in 2014,** and **Milia et al, in 2012)**.

In this study, human carious teeth have been utilized. The criteria for teeth selection was; carious lesion limited to occlusal surface as the rate of pulp exposure were of higher incidence on proximal surfaces than occlusal surfaces being of Si/Sta; 1.2 or 1.3 classification enabled having caries-affected dentin surrounded by normal dentin. Regarding the age of extraction, several studies showed that age did not greatly influence the dentin bond strength **(Kassa et al, in 2009,** and **De la Macorra and San-Nicolás, in 2005)**.

In the current study, teeth with chronic carious lesion have been selected. Chronic carious lesion is a slowly progressive lesion with intermittent periods of demineralization and remineralization, where remineralization of intertubular dentin, in

addition to crystalline precipitate formation in the dentinal tubules is in the advancing front of the demineralized zone. In slowly progressing lesion, caries-affected dentin acts as a defense mechanism providing some degree of natural barrier for the remaining dentin, thus the incidence rate of pulp exposure during excavation is moderate compared to acute carious lesion (**Banomyong et al, in 2009** and **Roberson et al, in 2006,)**.

Color and hardness are the renowned methods used to distinguish carious denting clinically. Evaluation of remaining denting hardness by tactile method is not proved to be a reliable or efficient method. And since, discoloration is also a subjective guide for the removal of caries-infected dentin, caries detecting dye is a more accurate method for caries-affected dentin detection. When the caries detecting dye is applied, the outer layer will be stained as the collagen fibers shows irreversibly breakdown, permitting more dye penetration of the solvent, this is followed by staining in the turbid layer that becomes lighter as it moves into the transparent zone and the sub transparent zone. However, potential problems have been associated with caries detecting dyes including the presence of remaining bacteria following the removal of the caries-infected dentin as these dyes appear to stain partial demineralized collagen matrices instead of cariogenic bacteria. Consequently, this may have causes to provide a 30% false positive diagnosis ehich is considered acceptable. In this study, Seek caries detecting dye was used, since it has been reported that it has no effect on the bond strength of dental composite (**Alleman and Magne, in 2012, De Almeida Neves et al, in 2011, Singh et al, in 2011, Hosoya et al, in 2008, Owens et al, in 2005,** and **Fusayama, in 1988**).

In this study, a confirmatory test to select caries-affected dentin as a substrate, dye permeability test was used**).** In this test, 10% methelyne blue was injected under pressure through the pulp chamber to stain normal dentin rather than caries-affected dentin. The low permeability of caries-affected denting in respect to normal dentin may have caused this accurate occurred staining, besides the presence of peritubular and intertubular crystals formation into the dentinal tubules. The dye permeability test was used to homogenize the tested specimens as much as possible. In addition, it was reported that the methylene blue used in the dye permeability test has no effect on bond strength of adhesive systems to dentin (**Mobarak and El-Badrawy, in 2011**).

Most in-vitro bond strength studies use flat dentin surfaces to test the bonding effectiveness of dental adhesive systems because when bonded restorations are applied in the cavities, they are subjected to higher polymerization contraction stresses at the interface. These stresses put the resin-tooth interfaces under sever tension during the critical setting of the adhesive, particularly when restoring cavities with high C-factor. Moreover, the caries-affected dentin and normal dentin were prepared on the same flat dentin surface, since irregular flat surface will significantly affect the homogeneity of stresses (**Armstrong et al, in 2010, Hebling et al, in 2007,** and **Feilzer et al, in 1987**).

It is widely known that two methods are generally used to assess the bond strength of dental adhesives; they are the micro-tensile and micro-shear bond tests. In the current study, the micro-shear bond strength test was selected, on the basis that it has proved to have relatively simple test preparations and better suitability to bonding tests (**El Zohairy et al, in 2010,** and **Burrow et al, in 2002**).

This study was carried out to assess the micro-shear bond strength of etch-and-rinse adhesive system and Universal adhesive system bonded to ND and CAD.

In the current study, single bond universal adhesive system (20.0±96.7MPa) showed statistically significantly lower mean µSBS than Adper Scotchbond Multipurpose adhesive

systems (22.0±10.4MPa) (*P*=0.008). This was in agreement with **Muñoz et al, in 2013,** who reported that the bond strength of the new category of universal adhesives bonded to dentin were inferior in comparison to etch-and-rinse or self-etch strategies. Possible explanation is that dentin is a challenging substrate for adhesion and that the universal adhesives are comprised of a heterogeneous composition that mixes various different components into the same solution (e.g., acidic and non-acidic monomers, solvents, fillers, initiators, and silane), the presence of these chemicals combined together in the Single bond Universal adhesive may have contribute to the decreased bonding strength to dentin. The three-step total etch, on the other hand, has a less complex composition than Universal adhesives, thus allowing satisfactory adhesion. However, **Isolan et al, in 2014,** contradicted the results of the present study ,they found that the bond strength of Universal adhesive to dentin showed similar results compared to etch and rinse or self etch adhesives tested. Also, these results contradicted with **Yoshida et al, in 2014**, who found that due to the fact that the single bond universal contains MDP, then it showed higher bond strength than Prime and Bond NT total etch adhesive on sound dentin.

In this study, micro-shear bond strength (μSBS) of ND substrate (24.3±8.4 MPa) showed higher statistically significant values than that of CAD (19.4±7.8 MPa) (*P*=0.001*). Regarding both adhesives when bonded to caries-affected dentin, the multipurpose total etch adhesive system showed statistically significantly higher micro-shear bond strength than Universal adhesive system and also microshear bond strength of normal dentin was higher than that of caries-affected dentin. The results was in agreement with **Müller et al, in 2017**, who found that, as the collagen fibrils are degraded by carious process and dentinal tubules distribution are also modified and this resulted in non uniform penetration of adhesive monomers and this exactly

what happened with single Bond universal as it contains acidic conditioners that is not sufficiently strong enough to etch sclerotic dentin. Also, these results was in accordance to **Erhardt et al, in 2008, Oskoee et al, in 2008, Wei et al, in 2008,** and **Pereira et al, in 2006,** who found that the μSBS of diverse bonding systems to normal or sound dentin was higher than that to caries-affected dentin. This could be explained that CAD may contain substances that interfere with free radical generation or propagation, leading to poor polymerization of adhesive monomers. It is reported that the degree of conversion of adhesive agent that penetrated the etched dentin in the CAD was lower than in the ND. Furthermore, the deposition of minerals in the transparent layer of dentinal tubules are highly acid resistant and may interfere with the infiltration of the resin monomer peripheral to dentinal tubules, leading to lower bond strength. On the other hand, the results of this study contradicted with those reported by **Scholtanus et al, in 2010,** and **Tosun et al, in 2008,** who reported higher bond strength of CAD than ND. The possible explanation of this finding is that the lower dentin permeability of CAD may therefore reduce water penetration into the interface, leading to long-term stability of the bond strength. On the other hand, **Arrais et al, in 2004,** found that bond strength of both CAD and ND are similar. Difference in results could be contributed to the difference in the technique of caries and operator variability.

CONCLUSIONS

The type of substrate affects the bonding performance of restoration in dentistry. While the chemistry and composition of the dental substrate may necessitate the use of specific adhesive materials protocols or strategies whether total etch three step, two step, self etch two step or one step that suites the condition of such substrate and hence, resulted in the increasing bonding durability of the restorations.

CLINICAL RECOMMENDATIONS

It is advised to make clinical trials using selective demineralization technique in which application of total etch beyond universal adhesive bonding may enhance bond strength to normal and CAD rather than self etch mode used.

Other studies should be made using storage media to stimulate oral conditions as this may change results of such context.

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