

Microleakage and Penetration Ability of Resin Sealant Versus Bonding System When Applied Following Contamination

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Abstract

Purpose: The purpose of this study was to evaluate the microleakage and penetration ability of sealing materials applied under different conditions of contamination.

Methods: One hundred twenty extracted human molar teeth were randomly assigned to 12 groups. The treatment groups were defined by the combination of sealing materials (Concise; Optibond system; Optibond system plus Concise) and 4 surface conditions (no moisture and no saliva contamination; moisture contamination; dried saliva contamination; wet saliva contamination). Each tooth was subjected to thermal cycling (5,000 cycles at 5°C-55°C) with a dwell time of 30 seconds and dye immersion (5% methylene blue for 24 hours). Microleakage, penetration ability, and fissure types were examined after sectioning. Multiple regression analyses and the Tukey test were used for statistical analysis.

Results: Concise showed significantly less microleakage than the Optibond system ($P<.031$) when the procedures were performed under no contamination or moisture contamination. However, when Concise was applied on the wet saliva-contaminated surfaces, considerably higher microleakage and unfilled areas were found compared to the use of Optibond alone or Optibond with Concise ($P<.001$).

Conclusions: When there is saliva contamination, the use of Optibond alone or with Concise is beneficial for decreasing microleakage and increasing the penetration ability of sealants. (*Pediatr Dent.* 2003;25:505-511)

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Epidemiological data show that, in most developed countries, the prevalence of dental caries has declined in recent decades.¹ However, there is current evidence that occlusal caries constitutes an increasing proportion of children's caries occurrence. Numerous clinical studies have documented the efficacy of pit and fissure sealants in caries prevention.^{2,3}

There is no doubt that sealant retention is the main determinant in maintaining a caries-preventive effect. Perhaps as much as dental procedures, however, sealant retention depends on meticulous operator technique.⁴ The greatest risk of sealant failure occurs soon after tooth eruption, when an operculum of gingiva is extended over the

distal marginal ridge of the tooth, thereby risking the occlusal surfaces to be contaminated at the time of sealant application.⁵ In fact, during the eruption period, many molar fissures begin the early process of carious breakdown. In addition, sealant failure rates on buccal and lingual fissures of molars are higher than those on occlusal surfaces due to inadvertent moisture contamination.⁶ Rubber dam application is generally not easy with children without local anesthesia. Cotton roll isolation makes it a practical necessity that an assistant be involved to provide 4-hand dentistry. Even with cotton rolls in place, it is easy for a child to contaminate the tooth while swallowing or with tongue movement.⁴

Table 1. Sealing Materials and Surface Conditions Used in Experimental Groups 1 to 12 (10 Teeth in Each Group)

Materials	Surface conditions			
	No contamination	Moisture contamination	Saliva contamination, dried off	Saliva contamination left undisturbed (wet)
Concise	Group 1	Group 2	Group 3	Group 4
Optibond	Group 5	Group 6	Group 7	Group 8
Optibond+Concise	Group 9	Group 10	Group 11	Group 12

Current sealant materials are unable to tolerate even minute amounts of contamination.⁷ Recently, a report by Fritz et al indicated that the 1-bottle adhesive system (of the so-called fifth generation) for use with the total-etch system was relatively insensitive to salivary contamination; as a rule, no re-etching was required.⁸

A dentine bonding system has bifunctional molecules with:

1. a methacrylate group that bonds to the restorative resin by chemical interaction;
2. a functional group that is able to penetrate wet dentine surfaces.

Additionally, the primer, in particular the acetone- or ethanol-based products, may tolerate saliva film reaching the underlying hydroxyapatite or collagen for firm mechanical bonding.⁹⁻¹⁰ It was hypothesized that these agents would be an effective adhesive when bonding to wet enamel surfaces as well. Recent work on improving bonding of sealants to saliva-contaminated enamel may help improve clinician confidence in sealant success, especially in difficult clinical situations (ie, newly erupted molars and buccal or lingual fissures). The use of dentine bonding agents (primer and adhesive) as an intermediate layer between the teeth and fissure sealants would be beneficial for increasing bond strength,¹¹⁻¹³ decreasing microleakage,¹³ and increasing the retention rate.^{6,14} An alternative available to prevent sealant failure is the application of a dentine bonding agent as the sole material for sealing fissure.

A study by Witzel et al indicated that the Optibond system, used as a single material for fissure sealing, had the best performance with similar results for both contaminated and dry-etched surfaces.¹⁵ However, conflicting findings have been published concerning the need of a dentine bonding agent.¹⁶ It was found that the use of a bonding agent prior to the application of a fissure sealant did not increase the retention rate.

Therefore, the present study was designed to compare the microleakage and penetration ability of a sealant, a bonding system, and a sealant with a bonding system applied under the conditions of contamination.

Methods

One hundred twenty extracted human third molar teeth free of caries, fluorosis, fissure sealants, and restorations, which had previously been stored in chloramine 1%, were

selected by visual inspection and using Diagnodent (KaVo, Biberach, Germany).¹⁷ The teeth were then randomly numbered from 1 to 120 and assigned to 12 treatment groups with 10 teeth each.

The treatment groups were defined by the combination of 3 sealing materials and 4 surface conditions. The 3 sealing materials were as follows:

1. Concise (unfilled white sealant, light cured, 3M Espe Dental Products, St Paul, Minn).
2. The Optibond system (hydrophilic primer and 48% filler adhesive, Kerr Manufacturing Co, Romulus, Mich).
3. The Optibond system plus Concise.

The surface conditions were described as follows:

1. no moisture and no saliva contamination;
 2. moisture contamination;
 3. saliva contamination and dried off;
 4. saliva contamination and left undisturbed (wet).
- All procedures were performed under room temperature (23 °C±2 °C). The treatment groups are shown in Table 1.

The steps used for all procedures were standardized as follows:

1. Clean using a bristle brush with nonfluoridated paste for 15 seconds.
2. Rinse 20 seconds with an air-water syringe.
3. Dry with oil-free compressed air for 15 seconds.
4. Etch using a 35% phosphoric acid gel (Ultra Etch, Ultradent Products Inc) for 60 seconds. During the etching process, the etchants were moved on the occlusal surfaces, leading the enhancement of the penetration ability of an etchant into the fissure systems.¹⁸
5. Rinse 30 seconds with an air-water syringe.
6. Dry with oil-free compressed air for 15 seconds.
7. Variable surface conditions:
 - a. No moisture and no saliva contamination: To serve as a control, the procedures for groups 1, 5, and 9 were performed in ambient room condition (relative humidity=40%±5 %).
 - b. Moisture contamination: The procedures for groups 2, 6, and 10 were performed in a humidity chamber (relative humidity=90%±2 %). To simulate oral condition, the etched enamel surfaces were placed in this chamber for 1 minute prior to sealant placement.
 - c. Saliva contamination and air-dried (groups 3, 7, and 11): Fresh whole saliva was collected daily from the principle examiner and syringed onto etched enamel surfaces until a film covered the entire enamel surfaces. This was left undisturbed for 10 seconds prior to drying with oil-free compressed air for 5 seconds.
 - d. Saliva contamination and left undisturbed (wet; groups 4, 8, and 12): Fresh whole saliva was syringed

onto occlusal surfaces and left undisturbed for 10 seconds. The excess saliva was not air-dried; it was blotted with a small sponge leaving a moist enamel surface before continuing with the procedure.

8. Sealant application:
 - a. Groups 1 to 4: Concise was applied using a microbrush and an explorer without loading the surfaces and cured for 40 seconds using the Astralis 7 (750 mW/cm², Vivadent, Schaan, Liechtenstein).
 - b. Groups 5 to 8: The Optibond system was used as a sole sealing material. A primer was applied using a light brushing motion for 10 seconds to help evaporate the solvent. Afterward, a bonding agent was applied and light-cured for 20 seconds using the Astralis 7.
 - c. Groups 9 to 12 (the Optibond system plus Concise): The Optibond system was used as an intermediate bonding agent under a fissure sealant. The primer was applied using a light brushing motion for 10 seconds to help evaporate the solvent. The bonding agent was consecutively applied and light-cured for 20 seconds using an Astralis 7. After that, Concise was applied on the cured Optibond using the same procedure as aforementioned in 8a. Care was taken not to place too much sealant material on each occlusal surface.

Thermocycling and dye penetration

Following sealant placement, the teeth were thermocycled in water for 5,000 cycles between 5°C±2°C and 55°C±2°C with a dwell time of 30 seconds. The surfaces of the teeth were then coated with melted utility wax, leaving the sealant and approximately 1.5 mm uncovered around the sealant. The coated teeth were immersed in 5% methylene blue for 24 hours to allow dye penetration into possible gaps between the tooth substance and the sealant.

Microscopic examination

For further examination, the wax coatings were stripped off and the teeth were rinsed thoroughly with tap water and embedded in self-curing resin to prevent chipping of the material. The teeth were then sectioned into 4 fragments with 3 parallel cuts in the bucco-lingual direction with a low-speed saw (Isomet, Buehler, USA). The thickness of the 4 sections per tooth was equal, and 6 sectioned surfaces were obtained from each tooth. Fissure type,

microleakage, and penetration ability were evaluated using a light microscope, at a magnification of ×25 (Wild, Leitz Ltd, Heerbrugg, Switzerland), equipped with a video camera linked to the computer. The examiner was blind to the groups. Optimas software (BioScan, Inc, Washington, DC) was used to measure the length of dye penetration, the enamel-sealant interface (mm), and the unfilled area of fissures (mm²).

Examination of fissure type

The micromorphological types of the fissure system were classified as follows¹⁹ (Figure 1): (1) U type; (2) V type; (3) Y1 type; and (4) Y2 type.

Examination of microleakage and penetration ability

The dye penetration value per sectioned surface was evaluated as shown in Figure 2. Three parameters were evaluated following Zyskind et al.²⁰

Lack of vertical sealant adaptation was identified by the presence of gaps between the sealant and the fissure wall or by the absence of sealant penetration into the fissures. The penetration ability was expressed as the unfilled area (mm²) of fissures (Figure 2).

Statistical analyses

The data were analyzed by general descriptive and multivariable methods using the general linear model procedure of the SYSTAT software for data analysis (SYSTAT, Inc, Evanston, Ill). Microleakage and penetration ability (dependent variables) were subjected to multiple regression analyses in order to test whether the independent variables (type of material, moisture and saliva contamination, and fissure type) influenced the performance of fissure sealants.

Table 2. Microleakage and Unfilled Area (Mean±SD) for Different Treatment Groups

Situation*	Material	Group	Microleakage (mean±SD)	Unfilled area (mean±SD)
No contamination	Concise	1	0.21±0.3	0.03±0.07
	Optibond	5	0.51±0.37	0.02±0.05
	Optibond+Concise	9	0.30±0.34	0.02±0.04
Moisture contamination	Concise	2	0.23±0.33	0.02±0.05
	Optibond	6	0.42±0.41	0.03±0.05
	Optibond+Concise	10	0.20±0.3	0.01±0.03
Saliva, dried off	Concise	3	0.24±0.27	0.02±0.04
	Optibond	7	0.51±0.37	0.03±0.05
	Optibond+Concise	11	0.42±0.38	0.02±0.03
Saliva, left undisturbed (wet)	Concise	4	0.91±0.25	0.10±0.15†
	Optibond	8	0.55±0.39	0.01±0.02
	Optibond+Concise	12	0.31±0.31	0.04±0.1

*Groups connected by a line are different at the 5% significant level within each condition of contamination (Tukey test).

†There were significant differences in unfilled areas between group 4 (Concise on wet contaminated surfaces) and all other groups (Tukey test).

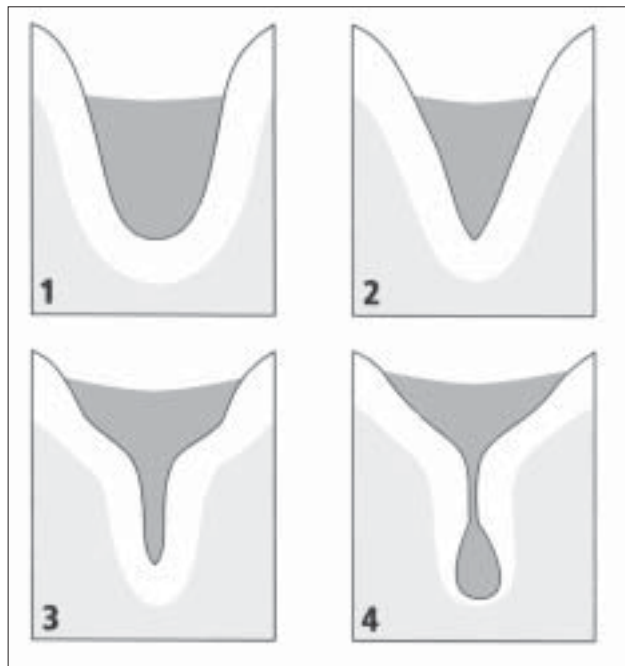


Figure 1. Diagrammatic depiction of the micromorphological types of fissure system. 1=U-type; 2=V-type; 3=Y1-type; 4=Y2-type.

Fissure type*	Proportion of microleakage (mean±SD)	Unfilled area (mm ²) (mean±SD)
U-type	0.48±0.4	0±0.01
V-type	0.34±0.39	0.01±0.03
Y1-type	0.37±0.36	0.05±0.09
Y2-type	0.33±0.38	0.09±0.09

*Groups connected by a line are different at the 5% significant level (Tukey test).

Dependent factor	Independent factors	df	F-ratio	P	R ²
Microleakage	Contamination	3	23.33	<.001	0.147
	Material	2	13.30	<.001	
	Fissure type	3	5.25	.001	
Penetration ability	Contamination	3	8.87	<.001	0.223
	Material	2	6.24	.002	
	Fissure type	3	49.78	<.001	

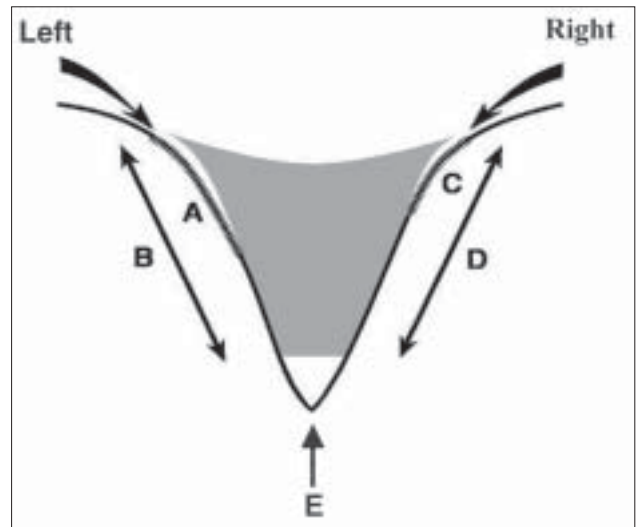


Figure 2. Scoring system employed for the evaluation of microleakage and penetration ability. A+C=length of dye penetration; B+D=length of sealant-tooth interface; $\frac{A+C}{B+D}$ =proportion of microleakage; E=unfilled area (mm²).

Differences between subgroups were checked for significance using Tukey's analysis. The level of significance was set at $P<.05$.

Intraexaminer reliability

Twelve teeth (10% of all teeth) were randomly selected and re-examined by the same examiner under the same conditions and using the same equipment. The intraexaminer reliability was tested using Cohen's unweighted kappa statistic (fissure type) and Spearman rank correlation coefficients (microleakage and penetration ability).

Results

Results from the duplicate examination showed that the intraexaminer reliability on fissure type as assessed by Cohen's unweighted kappa statistic was good ($\kappa=0.71$) and the intraexaminer reliability of the microleakage and penetration ability (unfilled area) as tested by Spearman rank correlation coefficients were 0.83 and 0.87, respectively (Systat 5.2.1, Systat Inc, Evanston, Ill).

The average proportion of microleakage for all groups is shown in Table 2. Statistical analyses showed the highest microleakage for Group 4 (Concise placed on wet saliva-contaminated surfaces). To compare specific groups, the Tukey test was used. This test incorporated an adjustment into the alpha level because more than 2 groups were being compared. Since group variances were unequal, the Tukey test version which allows differences in variances was used.

Although groups 2 and 3 (Concise with moisture and dried saliva contamination) exhibited slightly higher microleakage than Group 1 (control group), there were no significant differences in microleakage between the groups ($P>.05$). There were substantial differences in microleakage between group 4 (Concise with wet saliva contamination)

and all other groups. Conversely, when the Optibond system alone or the Optibond system with Concise were applied on the wet saliva-contaminated surfaces, they yielded significantly lower microleakage scores than using Concise alone ($P < .001$). Among the Optibond groups, there were no significant differences when performed under different conditions (groups 5-8; $P > .05$). However, the use of Optibond system as a sole sealing material (groups 5 and 6) under no contamination or moisture contamination exhibited a significant increase in microleakage compared to the use of Concise alone (groups 1 and 2; $P < .031$). In contrast, no significant differences were found in microleakage between the Optibond system plus Concise (groups 9-11) and the Concise groups (groups 1-3; $P > .61$).

As shown in Table 3, the average microleakage of U-type was significantly higher than that of other fissure types (V, Y1, Y2; $P < .004$). The multiple regression analyses revealed a significant impact of different materials, types of contamination, and fissure types on the microleakage of fissure sealants ($P < .001$; Table 4).

Data on the penetration ability (unfilled area) are summarized in Tables 2 and 3. The use of Concise with wet saliva-contaminated surfaces (Group 4) had considerably higher unfilled areas than the other groups ($P < .001$; Table 2). The use of the Optibond system alone or the Optibond system with Concise showed no significant differences in unfilled areas between different conditions of contamination ($P > .79$). As shown in Table 3, both Y1 and Y2 fissure types had significantly higher unfilled areas than U- and V-type ($P < .001$). The significant impact factors on penetration ability were fissure type, contamination, and material ($P < .002$; Table 4).

The pattern of microleakage and penetration ability (unfilled areas) was rather different. No correlation was found between microleakage and unfilled areas (mm^2) as tested by using Spearman correlation coefficients (0.041).

Discussion

Sealant effectiveness is directly related to its retention and is dependent on application procedures.^{2,21} It is generally accepted that adequate isolation is the most critical aspect of the sealant application process. Contamination of an etched enamel surface may have a deleterious effect on bonding.^{7,11} The present study was stimulated by recently published evidence that the efficacy of dentine bonding systems was not impaired by the presence of contamination.⁸ The authors hypothesized that when the dentine bonding systems were applied on the wet enamel surfaces, the development of a hydrophilic resin insensitive to moisture may increase successful sealant retention. Among the different sealing materials and bonding systems available, Optibond and Concise were used because of their favorable results compared to others.^{15,18,22}

In the present work, the results of the microleakage study indicated that Concise exhibited considerably higher

microleakage and unfilled areas than the Optibond system alone or with Concise when applied on wet saliva-contaminated enamel surfaces ($P < .001$; Table 2). This is in agreement with several other studies.^{11,12} The better results obtained from Optibond in wet contaminated surfaces could be attributed to several aspects. First, the Optibond system appeared to have a more hydrophilic nature than current sealant material due to lesser sensitivity to the contaminant of Optibond's primer (HEMA, ethanol, water). The ethanol-based products may have displaced the saliva from the occlusal surface, permitting the penetration of a bonding agent into the enamel porosities. In contrast to the hydrophobic characters of Concise, the resins were not able to penetrate into the plugged enamel porosities when enamel was wet, resulting in an insufficient number and length of resin tags to give adequate retention.²³

Although the use of the Optibond system under Concise showed slightly lower microleakage than the use of the Optibond system alone on wet contaminated surfaces, there were no significant differences in microleakage between these groups. It should be noted that in a real clinical situation, saliva contamination could occur at another step of procedure such as when a bonding agent was already cured. This might lead to a poor bond of a sealant to the cured bonding agent.

Accordingly, due to less time and material needed, the use of Optibond as a sole sealing material should be suggested as an alternative way when the ideal isolation is not possible. Therefore, the use of Optibond as a sealing material will make it possible to more effectively seal partially erupted teeth of patients with high susceptibility to caries, as well as teeth in which the buccal or palatal grooves approximate the gingival tissue. Furthermore, this alternative sealing material might be beneficial to patients who are not able to comply with rigorous isolation methods (ie, handicapped or very young patients).¹⁴ Additionally, when sealant preventive programs are performed under undesired conditions (eg, in mobile clinics or without an assistant providing 4-hand dentistry) the beneficial use of bonding systems might overcome the negative effect of contamination.

Nevertheless, the question remains whether the most susceptible tooth surfaces—the occlusal surfaces of the permanent molars—at an early stage of eruption should be routinely sealed with a sealing material which is insensitive to contamination. A long-term clinical trial should be conducted to compare the caries-preventive effectiveness of this technique and other caries-preventive regimes such as fluoride application, particularly in children with high susceptibility to caries.

However, when no saliva contamination is apparent, Concise displayed significantly less microleakage compared to Optibond. There may be many reasons for greater leakage in the Optibond groups. The amount of a primer after

applying and how moist the enamel surfaces are after drying are variable from each application and each operator.

The issues of the need for air drying and a proper drying time after primer application are still controversial. The application of the Optibond system might cause varied proportions of primer and adhesive in 1 application and varied thickness of an adhesive layer. In contrast to the single application of Concise, the 2-step application of Optibond might cause unintended bubbles due to the need for mixing and scrubbing steps between adhesive and primer. Further investigation is needed to reduce the technical sensitivity of a bonding system regardless of its bond to a restoration or a fissure sealant. In addition, various bonding systems available on the market contain distinct constituents that may result in dissimilar bonding potentials. As a consequence, when the proper isolation is possible, Concise is still recommended as a standard sealing material due to its better sealing ability, the fewer steps of application required, and the less time needed.

The present results indicated no significantly negative effect of the dried saliva contamination on the quality of sealing with Concise. This finding is not in accordance with previous work that showed that saliva contamination of etched enamel, even for a single second, resulted in a tenacious coating which blocked the porosities of etched enamel.⁷ The reason for the authors' results is not yet fully understood.

First, it could be that the bond quality of Concise might be not completely impaired by the exposure of etched enamel to saliva contamination for a few seconds. Likewise, when the etched enamel surfaces were contaminated for less than 10 seconds and were later dried off, the resin might possibly penetrate to the etched enamel surfaces which were partially plugged with the dried film of saliva, leading to sufficiently firm bonding. Secondly, thermocycling was found to be an effective way to mimic aging for sealant microleakage studies.¹⁵

However, different testing substances (several dye agents and varied numbers of thermal cycles performed) make comparison between studies difficult. Cohesive fractures were most frequently found with uncontaminated specimens, whereas contaminated specimens were fractured at the enamel-sealant interfaces.²³ Compared to other studies (500-1,000 thermal cycling),^{10,13} the relatively longer thermocycling (5,000 cycles) in the authors' study might cause more cohesive fractures in the uncontaminated specimens, probably leading to the penetration of dyes through the fractures. Consequently, a deep blue deposit (the precipitation of methylene blue) could be visualized in the sealant-tooth interface, resulting in seemingly higher microleakage of the control groups. Therefore, the significant differences in microleakage between the noncontaminated surfaces and the dried saliva-contaminated surfaces were not found.

As can be seen in Table 4, the proportion of microleakage was significantly related to the fissure type ($P=.001$). In the present work, care was taken not to place too much material on each occlusal surface.¹⁸ The sealing level of all specimens was supposed to be limited to half of the inclination of the cusps. Generally, shallow fissures (U-type) which lack fissure clefts at the fissure base had the shorter length of sealant-tooth interface than other fissure types (V, Y1, Y2), whereas the length of dye penetration of U-type was rather similar to one of the other fissures. Accordingly, the U fissure type might have a tendency to exhibit the higher proportion of microleakage than other fissure types.

Fissure depth and complexity is one of the great effects on the penetration ability of sealing materials (Table 4). It was conceivable that the deep pits and fissures, which are susceptible to caries attack, seem to be difficult to achieve a perfect seal. However, no correlation was found between the extent of leakage and the occurrence of unfilled areas at the sealant-tooth interfaces. Possibly, the value of total fissure obturation may not be significant if retention is gained from good adaptation of resin to the fissure orifice and adjacent cuspal inclines.²⁴⁻²⁵

It should be noted that this was an in vitro study. Further clinical study is needed to confirm these findings.

Conclusions

1. When there is no saliva contamination, Concise showed significantly less microleakage than the Optibond system.
2. When saliva contamination is apparent, the use of Optibond as a single sealing material or as an intermediate layer between enamel and sealant is beneficial for decreasing microleakage and increasing the penetration ability of sealants.

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