Bond strength and interfacial morphology of two adhesive systems to deciduous dentin: *in vitro* study

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This study evaluated the bond strength and the interfacial morphology of composite restorations (SMP: Scotchbond Multi-Purpose - 3M; CSE: Clearfil SE Bond - Kuraray) in primary dentin. There is no statistical difference between groups. Fracture modes revealed a weak zone in the demineralized dentin (SMP) and in the hybrid layer (CSE). The interfacial morphology was similar in both groups. The demineralized zone in bond structures has a negative influence in adhesive restorations over the time.

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INTRODUCTION

To improve the quality of the adhesive restorations, the resin monomers must be diffused into the demineralized dentin. A functional hybrid layer should be large enough to allow a stable interlocking of the bonding agent around the exposed collagen network.

Dissolution of the mineral component is the most important modification that occurs on the dentin surface when etching. Previous studies have showed the differences between deciduous and permanent teeth in dentinal composition[1,2] and morphology.[3]

Regardless of these differences, the same etching protocol has been recommended for either deciduous or permanent teeth adhesion. This fact causes the establishment of a different tooth – composite interface in both primary and permanent teeth.[4, 5] When strong acids are used for long periods of time in primary dentin, a demineralized dentin zone is created within the bonding structure by incomplete monomers diffusion into collagen network. The lack of full penetration has a negative influence on bond integrity.[6]

Self-etching primers were developed to simplify the bonding procedure and are based on the use of non-rinsed acidic polymerizable monomers, which serve as conditioner, primer and adhesive.[7] These materials have been strategically used to prevent the occurrence of the demineralized dentin collapse. The acidic monomers act through smear layer to the subjacent dentin, incorporating it in the union process.[8] Theoretically, this prevents the excessive lost of dentin matrix and solubilizes apatite crystals around the collagen network, permitting the good infiltration of the adhesive monomer in the substrate. In an adhesive system, which simultaneously demineralizes dentin and infiltrates it with monomers, no gaps would be left between the primed dentin and the demineralization front of the underlying dentin matrix.[9]

Taking into account that the information about the use of the latest generation of bonding systems for deciduous teeth is still limited, the present study aims to evaluate the bond strength and interfacial morphology provided by two bonding systems. One bonding system with total etch technique, and one other self-etching primer, used in dentin of deciduous teeth. The results were related to these bond strengths with the distribution of failure modes as observed by SEM.

MATERIALS AND METHODS

Eighteen sound primary molar teeth were selected (first and second; upper and lower) with physiological advanced reabsorption, or extracted for orthodontic reasons, in patients from ten to twelve years old, both

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male and female, who were under care in the Pediatric Dentistry unit at Federal University of Rio Grande do Sul - Brazil (UFRGS). Informed consent was obtained from all subjects under a protocol approved by the UFRGS review board.

Standard occluso-proximal (class II) vertical slots cavities (3mm buccal-lingually, 2mm proximal-axially and cervical limit 1mm beneath the enamel – cement junction) were prepared with a diamond point (no. 2094) in a high speed hand piece with air and water spray coolant. Each tooth received two occluso-proximal (occluso-mesial / occluso-distal) cavity preparations.

A hybrid resin composite (Filtek Z 250 – 3M) and two bonding systems (Scotchbond Multi-Purpose – 3M / Clearfil SE Bond – Kuraray) were selected to the study. Each bonding agent was applied using the instructions of the manufacture to one of the occluso-proximal cavity preparation of the same tooth. For the Scotchbond Multi-Purpose system, an acid etching (Acigel –SS White/phosphoric acid 10%) was previously applied.

The cavities were filled with resin composite by incremental technique. The first layer inserted horizontally at the cervical portion and, the other two increments, inserted vertically and each one light-cured with 500mw/cm^2 for 20 seconds, using a visible light source (XL 2500 – 3M).

The restored teeth were stored in distilled water at 4ºC for one week and sectioned buccal – lingually with a diamond saw to separate the class II restorations.

The resin-filled cavities were sectioned perpendicular to the adhesive interface formed in the axial wall by means of a diamond saw with a water coolant. One or two resin-dentin bonded sections (thickness – 0.7 mm) were obtained for tooth. These slices were carefully trimmed and shaped with a superfine diamond point (no. 3122 FF) to produce an hourglass shape to form a gentle curve along the bonded interface (constriction width 1.0 mm) from both sides until 0.7mm? of bonded surface remained. These specimens were then attached to a testing apparatus with a cyanoacrylate adhesive, and a tensile load was applied by a tester machine (Instron 441 testing machine-Instron, USA) at a crosshead speed of 0.5mm/min. The bond strength values obtained in the rupture of the specimens were registered (Kgf) and expressed in Mega Pascal (MPa). After the micro-tensile bond testing, all fractured portions of the specimens were stored at room temperature for 24 hours. The specimens were sputter-coated with gold for the analysis of the fracture pattern by scanning electron microscope (SEM-JEOL 5600LV, Tokyo, Japan) to determine the most fragile zone of the union between the dentin and the restorative material

The types of fracture were classified (adapted from Hashimoto *et al.*[10]) into four different types. Type I failure was in the adhesive system/resin composite. Type II failure was in the top of the hybrid layer. Type III failure was in the demineralized dentin or bottom of the hybrid layer. Type IV was an assorted failure, which has the combination of more than one type of the previous failures.

Additionally, five slabs in each group were prepared to have the morphology of the resin-dentin interface characterized by SEM.

Each flat surface were polished by means of silicon carbide papers of decreasing abrasiveness (600-1200 grip) and soft cloths using alpha-alumina powder (6, 3, 1 and 0.25µm) suspended in distilled water, and after, cleaned in ultrasonic bath for ten minutes. After this, the specimens were immersed for 5 seconds in a 50% phosphoric acid solution, washed with distilled water and submerged for 15 minutes in a 10% NaOCl to remove the organic content. Followed by 24h desiccation at room temperature, the samples were then sputter-coated with gold and examined using a field-emission SEM.

The qualitative evaluation of the adhesive interface was based on the analysis related to the presence/absence of gaps, formation of the hybrid layer and resin tags. The results of the micro-tensile bond testing were submitted to a statistical analysis using the Student's *t*-test. The types of fracture were submitted to descriptive evaluation.

RESULTS

In a total of 36 occluso-proximal restoration made (18 for each group), 26 specimens were used for the micro-tensile bond testing, 11 for group 1 (SMP), and 15 for group 2 (CSE). Some specimens were lost during the methodological procedures.

The results of micro-tensile bond testing were submitted to analysis using Student's *t*-test, with the purpose to compare two averages from two independent groups (Table 1) and showed no statistical differences in bond strength among the two groups (p<0,05).

Table 1. Result of Student's t-test of the comparison of the two averages between the groups.

Group	*n*	*Average*	*sd*	*p*
SMP	11	21.84 Mpa	9.90 Mpa	0,27 (ns)
CSE	15	25.19 Mpa	5.33 Mpa	

DISCUSSION

The use of adhesive systems associated with hybrid composite has become frequent in the pediatric dentistry, either for deciduous or young permanents teeth. Nevertheless, there is little scientific information addressed to adhesion in deciduous dental substrate, mainly related to the use of self-etching primers.

In a SEM study, Nör *et al.*[4] observed that the adhesive interface formed in dentin of deciduous teeth was thicker when compared to permanent teeth, suggesting

a greater reactivity of deciduous dentin when etching. A similar situation has also been observed by Olmez *et al*.,[5] who asserted that this is the possible cause to explain the differences in bond strength found in deciduous and permanent teeth, related in previous studies in the literature.[11-14]

The increased thickness of the demineralized zone and the subsequent incomplete impregnation of the bonding system in the collagen network result in an area of collagen fibers without support, susceptible to the hydrophilic degradation and a consequent reduction of the adhesive strength over the time.[4, 6, 15]

This occurrence tends to be more frequent in adhesive restorations of deciduous teeth, due to the composition less mineralized and also because the same etching protocol for permanents is common used for deciduous teeth.

The self-etching primers have acidic monomers in its composition with higher pH values compared to traditional acids used. This characteristic make these materials not so aggressive in relation to the degree of demineralizing the dental substrate, and theoretically, the difference between the decalcificated/penetrated dentin by the bonding system would be limited, or even absent, because demineralization and the impregnation of the hydrophilic monomers simultaneously occur, incorporating the smear layer in the adhesion process.[8]

The present study used the micro-tensile method to measure the bond strength level provided by two bonding systems to the primary dentinal substrate. The statistical analysis revealed no statistical difference (p=0.27) between the two material tested (group 1: 21, 84MPa / group 2: 25, 19MPa). A possible explanation for these similar results would be supported by the application protocol of the Scotchbond Multi-Purpose (3M) in which it was used an acid etching less concentrated (10% phosphoric acid) and for a shorter period of time (7s in dentin) in relation to the recommendation of the manufacturer.

An important observation refers to the standard deviation of the results found for each bonding system (SMP ± 9.90MPa / CSE ± 5.33MPa). This variability may be support in two happenings. The first would be related to the protocol use of Scotchbond Multi-Purpose system that has more steps to accomplish the adhesive restorative procedure than the Clearfil SE Bond. According to the literature, the greater number of steps for the application of the adhesive system, most probability of incorporating errors during the restorative procedure.

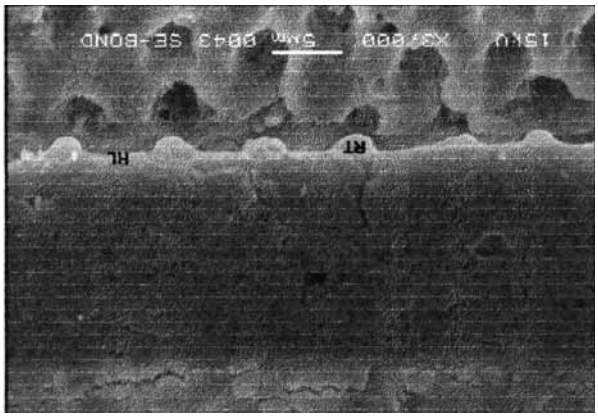
The second situation is related to the interaction of the material to the dental substrate. While the traditional systems, which use the acid etching previous to the adhesive application, remove totally the smear layer, the possibility of dehydrating in excess the demineralized dentin increases considerably. If that occurs, the collagen network collapse and the penetration and diffusion of the adhesive monomers are limited, making difficulty the formation of a uniform hybrid layer. On the other hand, in the self-etching systems, the smear layer is incorporated during the hybridization process, what decreases the risk of dentin dehydration, once the demineralized region is partially "protected" by this amorphous layer of dentin.

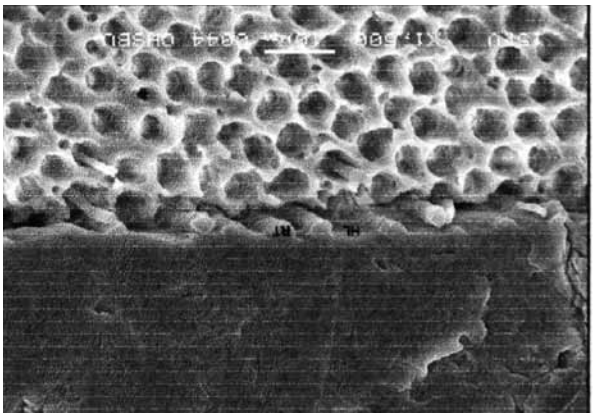
The mean values of bond strength observed in group 1 in this present study (SMP: 21.84±9.9MPa) was very closed to the control group - *in vitro* - (SMP: 28.3±11.3MPa) in the Hashimoto *et al*.[6] study, that evaluated the bond strength of occlusal and occluso-proximal of composite restoration in deciduous teeth aged in oral environment, and found a significant reduction of the bond strength over the time (1 to 2 years: 15.2±4.4MPa; 2 to 3 years: 9.1±5.1MPa). The analyses made by SEM of the fractured surface revealed, either in the *in vivo* or in the *in vitro* group, the presence of a demineralized dentin area, indicated by the presence of open spaces, originally occupied by collagen fibers, without the impregnation of restorative material in the dental tissue.

Agostini, *et al*.[16] evaluated the bond strength of tree self-etching primers (Prompt L-Pop – ESPE, Clearfil SE Bond – Kuraray, Etch and Prime 3.0 – Degussa) and one adhesive with total etch technique (Prime and Bond NT – Dentsplay/Caulk) to deciduous teeth. The tested materials bonded efficiently to deciduous enamel, but only the self-etching system Clearfil SE Bond (Kuraray) reached adequate scores of bond strength in dentin. A possible explanation of the results would be in the mineral contents reduced in dentin compared to enamel of deciduous teeth, what allowed a better interaction of the self etching system, because it has phosphated radicals with a weaker power of decalcification than the traditionally acid gels used.

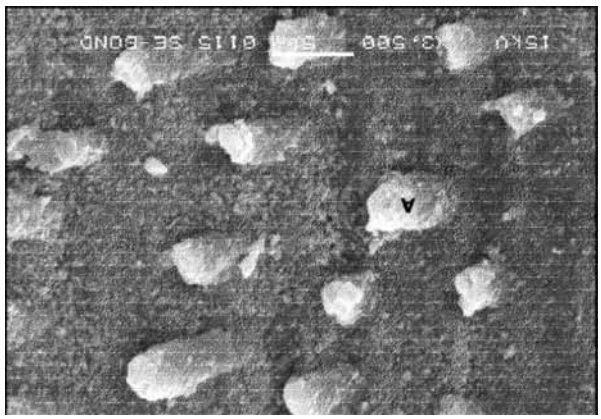
Thus, the bonding agent needs to penetrate and stay close to the dental structures. The utilization of SEM also represents an available resource to evaluate the interaction between the restoration material and the dental substrate.

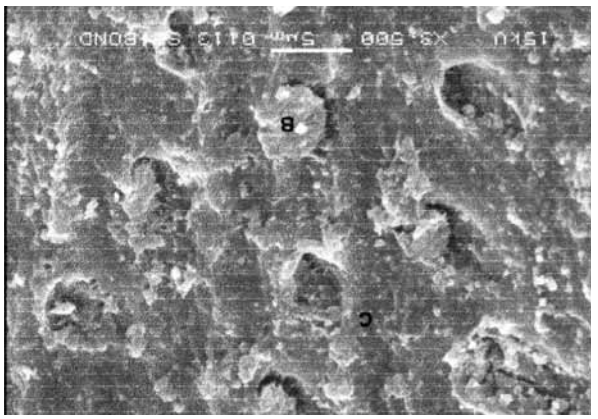
A relevant factor, during observation of the adhesive interface through SEM, is related to the methodology applied in the preparation of specimens for post visualization. The processing of the specimens for an analysis by SEM involves several steps, some of them can produce artifacts that conduct to misinterpretations about the interaction between the dental substrate and the restorative material. The presence of gaps, frequently observed in studies of adhesive interface, might occur not only in situations where there is a fragile union tooth/restoration, but also when the thickness of the section (that contains the interface) is too thin, what cases the breakage of the adhesive interface during the processes of cutting, polishing, dehydration, metallization and formation of vacuum for SEM analysis, and this negatively influences the evaluation.





Figures 1 and 2. Micro-morphologic pattern (1000x) of both sides of the fractured surfaces (group 1: SMP) in the micro-tensile bond testing showing the dentinal tubules region without composite remaining, representing failure in the demineralized dentin area (type III).





Figures 3 and 4. Photomicrographs (3500x) of the fractured sections (group 2: CSE) to the micro-tensile bond testing. It is possible to notice the presence of resin tags (A) in the section corresponding to the restorative material, and dentinal tublules filled with composite tags (B) in the homolog dentinal section. It can be observed the occurence of diagonal grooves (C) from the cavity preparation, representing failure in the top of the hybrid layer (type II).

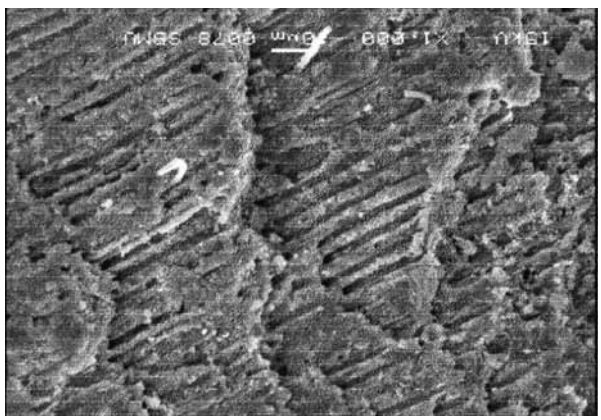


Figure 5. Photomicrograph (1500x representative of the union interface formed between dentin and Scotchbond Multi-Purpose adhesive system. There are an intimate relation of the restorative material with the dentin, presence of rein tags (RT) and the occurrence of the hybrid layer (HL).

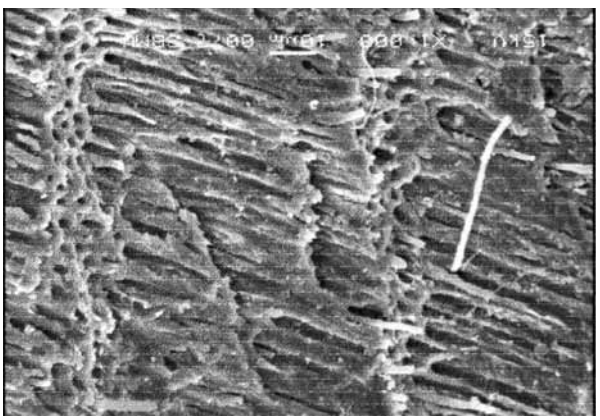


Figure 6. Micro-morphologic aspect of the composite/dentin union found in group 2 (Resin composite, RC; Adhesive system, AS; Hybrid layer, HL; Resin tags RT).

In the present study, gaps ere not observed gaps on the specimens submitted to the analysis of the adhesive interface. The formation of the hybrid layer with good adaptation to the dental structure was observed in both bonding systems used (Scotchbond Multi-Purpose – 3M / Clearfil SE Bond – Kuraray).

Telles *et al.*[17] observed by SEM the occurrence of

gaps in the adhesive interface when it was used the self-etching bonding system Prompt-L-Pop (ESPE), either for deciduous or permanent teeth, when used with resin composite, compomer or glass-ionomer cement.

Hashimoto *et al*.[18] evaluated the union interface formed on deciduous teeth after the utilization of the Scotchbond Multi-Purpose (3M) adhesive system. Samples *in vivo* and *in vitro* were prepared and analyzed by SEM. On those teeth that stayed in oral environment for certain period of time, the presence of a hybrid zone in dentin was visible, but the detection of gaps on the union interface was a very frequent happening. In addition, it was observed micro-morphological changes that suggest deflection of the collagen fibers and degradation of the structures involved in the adhesion. The pattern of the adhesive interface found for the control group (*in vitro*) showed the presence of the hybrid layer with several resin tags in the dentinal tubules and the absence of gaps. Micro-morphological evidences similar to those found in the group 1 and group 2 of the present study (Figures 5 and 6).

The resin/dentin interaction can also be evaluated by means of the type of fracture that occurs in the adhesive interface. Some studies that used fractographic (types of fractures) analysis, utilizing SEM and TEM, showed controversial outcomes indicating that the upper portion of the hybrid layer[19,20] and also the deepest zone, represented by the demineralized dentin area[21, 22] can be considered as the most fragile in the adhesive interface.

The mode of fracture observed seems to have a direct relation to the type of the adhesive used, probably related to the solvent present in the bonding agent. When a bonding system with acetone is applied, the fracture occurs mostly on the surfaces of the hybrid layer under tension. On the other hand, an adhesive system that uses water or alcohol is utilized, the origin of the fractures is on the demineralized dentin area. A possible explanation for this happening is that the bonding system with acetone allows a better connection between the collagen fibers and the composite, because of its potential to dislocate the water. A water/ethanol solvent, in contrast, can produce a weak or no interaction between the collagen fibers surface and the composite.[23]

The results of this present study partially agree with the observations made by Nakabayashi and Pashley[23] in relation to the findings related to the type of fracture (analyzed by SEM) that occurs from micro-tensile bond testing. The most prevalent failure pattern observed for the adhesive system Scotchbond Multi-Purpose (3M) was in the demineralized dentin region, which was not completely filled by the diffusion of the adhesive monomers. According to previous information, this type of fracture is often related to the solvent in the primer (water solution of a polialcenoic acid + Hema) present in this adhesive system.

On the other hand, in the self-etching system that has water as solvent, it was frequently observed a greater number of failures on the top of the hybrid layer proved by the presence of composite remains in the dental tubules in one of the sections of the fracture (Figure 3), and also because it presents resin tags in the homolog section (Figure 4). Moreover, the occurrence of grooves on the fractured surfaces represents the lines caused by the diamond point that was used to make the cavity preparation. Its presence suggests that the failure occurred on the top of the hybrid zone.

Considerations must be made in relation to the association between the results of bond strength obtained and the type of fracture observed. The smallest values generally were associated to the failure occurred in the demineralized dentin area (Table 2). This situation was exclusively found in the group 1 of the present study, in which the adhesive system requires the previous appli-

Table 2. Type of failure X group 1.

G1-SMP	MPa	Type of failure
S1	15.34	Type IV (DD/HL)
S2	13.22	Type III (DD)
S3	29.65	Type II (HL)
S4	46.07	Type I (AS/RC)
S5	27.1	Type IV (DD/AS)
S6	11.17	Type III (DD)
S7	23.76	Type II (HL)
S8	16.94	Type III (DD)
S9	21.61	Type II (HL)
S10	20.1	Type II (HL)
S11	15.26	Type III (DD)

Legend:
S = specimen
AS = adhesive system
RC = resin composite
HL = hybrid layer
DD = demineralized dentin

Values of bond strength in MPa obtained in the micro-tensile bond testing of the Scotchbond Multi-Purpose adhesive system applied to the dentin of deciduous teeth and types of failures of specimens analyzed by SEM.

Table 3. Type of failure X group 2.

G2-SMP	MPa	Type of failure
S1	17.38	Type I (AS/RC)
S2	24.39	Type I (AS/RC)
S3	26.44	Type II (HL)
S4	21.77	Type II (HL)
S5	34.06	Type II (HL)
S6	19.98	Type I (AS/RC)
S7	24.34	Type II (HL)
S8	32.24	Type II (HL)
S9	31.96	Type II (HL)
S10	32.32	Type II (HL)
S11	26.23	Type (AS/RC)
S12	22.39	Type II (HL)
S13	23.29	Type II (HL)
S14	23.22	Type II (HL)
S15	17.88	Type I (AS/RC)

Legend:
S = specimen
AS = adhesive system
RC = resin composite
HL = hybrid layer
DD = demineralized dentin

Values in MPa obtained in the micro-tensile bond testing of the Clearfil SE Bond adhesive system applied to the dentin of deciduous teeth and the modes of failures of specimens analyzed by SEM.

Table 4. Distribution of type of failures for groups.

	Group		
Type of failure	*SMP*	*CSE*	***Total***
Adhesive system/resin composite - type (1)	1 (9.0%)	5 (33.3%)	6
Hybrid layer - type (II)	4 (36.4%)	10 66.7%)	14
Demineralized dentin - tye (III)	4 (36.4%)	—	4
Association failures - type (IV)	2 18.2%)	—	2
Total	11	15	26

cation of the acid etching. Even using a less concentrated and for a shorter period of time than the recommended by the instructions of the manufacturer, it was not possible to avoid the differences between dentin decalcification/penetration of the adhesive primers. This condition has, over time, a negative repercussion into the bonding structures.[6, 18]

Group 2, represented by the self-etching primer system, presented more failures on the upper portion of the formed hybrid layer (type II) and the bond strength values more uniform distributed (Table 3). This kind of failure can be clinically more acceptable because the tubules are partially obliterated, protecting the subjacent dentin from eventual stimuli that might cause sensibility or, sometimes, demineralization caused by recurrent caries lesion in a patient with caries activity not controlled.

The absence of failures on the demineralized dentin in the group that used the self-etching system Clearfil SE Bond (Kuraray), simulates the performance of this kind of material in clinical studies, because it does not includes regions in which the demineralized dentin matrix was not involved by the adhesive material.

New materials are often introduced into the market, many of them without clinical trails. Researchers should pay attention to longitudinal studies, with the goal to understand the clinical events that may have repercussion on the restorations. Further knowledge in relation to stability of adhesion to the human dental substrate, as well as the sealing of the cavity margins area, is necessary to preview the performance of the adhesive restorations over the time in a controlled oral ambient, which surely will influence the clinic profile of the restoration.

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