



## Effect of Tubular Orientation on the Microtensile Bond Strength of Composite-dentin using Universal Bonding Agents

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### ABSTRACT

The aim of this study was to evaluate the effect of dentinal tubules orientation on the composite-dentin microtensile bond strength using universal adhesives. A total of 36 caries-free third molars were selected. The teeth were allocated to (axial, wall) and (occlusal, floor) groups and each group was divided into three subgroups based on the type of bonding used. The specimens were then sectioned to obtain dentin-composite sticks with a binding area of approximately 1 mm<sup>2</sup>. Then sticks were mounted in the universal testing machine (UTM) at a crosshead speed of 0.5mm/min. Data collection was carried out using two-way ANOVA and T-test. Then, the failure mode of the specimens was observed using a stereomicroscope, and one specimen from each group was evaluated under a scanning electron microscope (SEM). The results of two-way ANOVA showed that the highest and lowest mean microtensile bond strength ( $\mu$ TBS) were observed in the group of Adper Single Bond 2 adhesive (axial, wall) (28.92 $\pm$ 8.75) and Single Bond Universal adhesive (occlusal, floor) (12.83 $\pm$ 7.90), respectively. On the other hand, there was no significant difference between the three adhesives in the mean of  $\mu$ TBS in the (occlusal, floor) orientation, but the mean  $\mu$ TBS of Adper Single Bond 2 adhesive was significantly higher than that of G-premio and Single Bond Universal adhesives in the (axial, wall) orientation ( $P=0.05$ ). There was no significant difference in two universal adhesives ( $P=0.994$ ). The stereomicroscopic analysis showed that the highest adhesive failure was related to Adper Single Bond 2 in (axial, wall) orientation. However, the failure mode results were nearly identical in both adhesives in the (axial, wall) orientation. The failure mode results were also similar in all three adhesives in the (occlusal, floor) orientation. The dentinal tubules orientation showed no significant effect on the microtensile bond strength of the G-premio, Single Bond Universal adhesives. Adper Single Bond 2 had a significantly higher  $\mu$ TBS rate in the (axial, wall) orientation, but its  $\mu$ TBS was similar to that of the universal adhesives in the (occlusal, floor) orientation, which was not statistically significant.

**Key words:** Microtensile Bond Strength, Tubular Orientation, Universal Bonding Agents

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Dentin has a complex biological structure and dentinal tubules are main tissue structure of its [1-3]. Dentinal tubules run continuously from the dentin-enamel junction (DEJ) to the pulp in coronal dentin, and from the cementum-dentin junction (CEJ) to the pulp canal in the root and

### INTRODUCTION

their wider portion runs toward the pulp and occupies approximately 1% of the DEJ area and 22% of the area near the pulp [4]. Since the dentin permeability is related to dentinal tubules, the regional variation in the size and density of the tubules make definite changes on the dentin permeability [5]. The achievement of high and stable adhesion and bond strength between dental structure and restorative material is one of the important goals of the restorative dentistry [6]. For this purpose, dental adhesives are used to strengthen adhesion between composite resin and tooth structure [7]. The adhesion process depends on several factors, such as type of substrate, type of adhesive, the environment moisture, and the operator's ability to perform the bonding method [8]. Some of the substrate variables include dentin moisture and regional differences and tubules orientation [9-11]. When the adhesion process is perpendicular to the orientation of dentine surface (occlusion, floor), long and solid resin tags are formed, but the resin tag is not formed when the adhesion process is parallel to the dentinal tubules (axial, wall). On the other hand, the tubular fluid flow (TFF) exposed to the dentin surface seems to interfere with the quality of the dentinal adhesive interface and may reduce the resin-dentin bond strength [12]. Studies show that the orientation of dentinal tubules can have a major impact on physical properties of dentin, formation of resin tags and hybrid layer, and can affect the bonding process [12]. On the other hand, with the improvement in dental bonding systems, one-bottle simplified adhesives were developed to accelerate the adhesion process and greater satisfaction of clinician [13]. These new simplified adhesives are called universal, multipurpose or multi-mode adhesives [14, 15], which are less technique sensitive and user-friendly. Universal adhesives can be applied according to the etch & rinse (ER) and the self-etch (SE) strategies with one or two application step [16]. Studies have been conducted on the bond strength of universal adhesives, such as Miguel *et al.*, study (2013) [17], which was on the immediate bonding properties of the universal adhesives and the results showed that there was no significant difference between the  $\mu$ TBS of the peak universal adhesive with Clearfil SE Bond of the control group in the self-etch mode and Adper Single Bond 2 of the control group in the Etch & rinse mode; however, Scotchbond Universal and All-Bond Universal adhesives showed a decrease in  $\mu$ TBS considering the respective control groups. Poggio *et al.*, [18] also performed a study on the effect of dentin

preparation on bond strength of universal adhesives, which included five different universal adhesives, including Futurabond M +, Scotchbond Universal, Clearfil Universal Bond, G-Premio Bond and Peak Universal Bond. The results of this study showed that the type of universal adhesive has no significant effect on the shear bond strength of the composite resin. On the other hand, few studies have been conducted to evaluate effect of tubular orientation of the dentine on the  $\mu$ TBS of universal bonding systems. Therefore, the aim of the present study was to evaluate effect of tubular orientation of the dentine on the composite-dentin  $\mu$ TBS using universal adhesives.

#### Null hypothesis

The universal adhesives showed different early  $\mu$ TBS in the parallel and perpendicular orientation of dentinal tubules.

### MATERIALS AND METHODS

#### Teeth preparation

A total of 36 caries-free third molars, which had been extracted within the three last months for various reasons, were selected, cleaned, and stored in 10% formalin solution. 24 h before the study placed in the distilled water. The occlusal surface enamel of each molar was ground away using an orthodontic trimmer (Pars medical Co., Tehran, Iran) perpendicular to the longitudinal axis of the tooth to achieve a flat dentin surface lacking any residual enamel. To create a uniform smear layer, dental surfaces then wet-ground with #600 SiC paper (19). Then, the teeth were randomly divided into two equal groups as follows (Figure 1) (N= 18).

Group axial or wall [W]; teeth were sectioned along the longitudinal axis using the disk in such way that the axial dentin was exposed, then the cutting surface was considered as the surface of the specimen and 2 specimens were obtained from each tooth. Group occlusal or floor [F]; teeth were sectioned perpendicular to the longitudinal axis using the disk in such way that the occlusal dentin was exposed, then the cutting surface was considered as the surface of the specimen and 2 specimens were obtained from each tooth.

Then each of the groups was divided into three subgroups based on the type of bonding used as follows (N=12):

1. The FS group: Two layers of Adper Single Bond 2 adhesive were applied to the exposed occlusal

dentin surface using micro-brush according to the manufacturer’s instruction, and then cured by a LED Light Curing Device (Kerr, Orange, CA, USA) with an output of 1200 mW / cm<sup>2</sup>.

2. FG Group: One layer of G-premio Bond / GC adhesive was applied on the surface of the occlusal exposed dentin surface using a micro-brush in self-etch mode according to the manufacturer’s instruction and then cured by the curing device similar to the previous group.

3. FSu Group: One layer of Single Bond Universal/3M adhesive was applied on the occlusal exposed dentin surface using a micro-brush in the self-etch mode according to the manufacture’s instruction and then cured by the curing device similar to the previous group.

4. The WS group: Two layers of Adper Single Bond 2 adhesive was applied on the axial exposed dentin surface using a micro-brush according to the manufacture’s instruction, and then cured by the curing device similar to the previous group.

5. WG Group: One layer of G-premio Bond / GC adhesive was applied on the axial exposed dentin surface in the self-etch mode using a micro-brush according to the manufacture’s instruction and then cured by the curing device similar to the previous group.

6. WSu group: One layer of Single Bond Universal / 3M adhesive was applied on the axial exposed dentin surface in the self-etch mode using a micro-brush according to the manufacture’s instruction, and then cured by the curing device similar to the previous group.

Then, the X-tra fill (universal shade) composite (Voco GmbH, Cuxhaven, Germany) with a thickness of 4 mm was placed on the surface and cured with the aid of a LED (Power= 1200 mw / cm<sup>2</sup>) for 40 seconds [34]. Table 1 shows the materials used and their application procedure in the present study. The specimens were then kept in distilled water for 24 hours at room temperature.

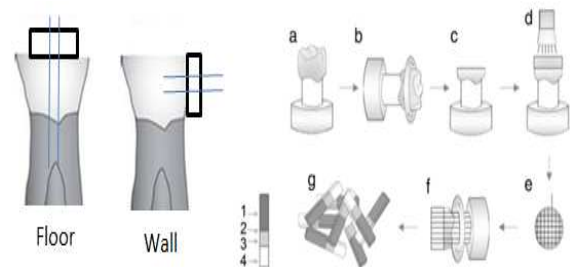


Figure 1: 12 sticks obtained from each molar for each group

Table 1: The materials used and their application procedure in the present study

Material	PH	Composition	Application
G-Premio Bond (GPB) GC Corp. Tokyo, Japan P	1.5	10-MDP, phosphoric acid ester monomer, dimethacrylate, 4-MET, MEPS, acetone, silicon dioxide, initiators	1. Apply using a microbrush 2. Leave undisturbed for 10 s after application 3. Dry thoroughly for 5 s with oil free air under maximum air pressure 4. Light cure for 10 s
Single Bond Universal 3M ESPE, St Paul, MN, USA	2.7	MDP phosphate monomer, dimethacrylate resins, HEMA, Vitrebond™ Copolymer, filler, ethanol, water, initiators, silane	1. Apply the adhesive on the surface and rub it in for 20 s 2. Gently air-dry the adhesive for approximately 5 s for the solvent to evaporate 3. Light cure for 10 s
Adper Single Bond 2 (3M ESPE, St Paul, MN, USA)	0.6	1. Etchant: 35% phosphoric acid (Scotchbond Etchant) 2. Adhesive: bis-GMA, HEMA, dimethacrylates, ethanol, water, photoinitiator, methacrylate functional copolymer of polyacrylic and poly(itaconic) acids, 10% by weight of 5 nm-diameter spherical silica particles	1. Apply etchant for 15 s 2. Rinse for 10 s 3. Blot excess water 4. Apply 2-3 consecutive coats of adhesive for 15 s with gentle agitation 5. Gently air dry for 5 s 6. Light polymerize for 10 s at 1200 mW/cm <sup>2</sup>
X-tra fil (bulk-fill) Micro-hybrid U Voco, Guxhaven, Germany.		Resin matrix: Bis-GMA, UDMA, TEGDMA Filler type: Bariumboronealuminosilicate glass (2e3 mm) Filler(W %):86	Maximum depth :4 mm 10-sec curing >1000mW/cm <sup>2</sup> Viscosities :Regular

Bis-GMA: bisphenol glycidyl methacrylate; HEMA: 2-hydroxyethyl methacrylate; MDP: methacryloyloxydecyl dihydrogen phosphate; 4-MET: 4 methacryloyxyethyltrimellitate anhydride; MEPS: Methacryloyloxyalkyl thiophosphate.

**Table 2: The similar letters are not statistically significant**

BONDING	SURFACE	N	MEAN± SD	Min	Max
Gpremio bond	FG	12	18.01±9.12 <sup>a</sup>	9.50	42.70
	WG	12	15.64±6.21 <sup>a</sup>	5.70	26.30
Single Bond universal	FSc	12	12.83±7.90 <sup>a</sup>	3.20	25.30
	WSc	12	15.25±3.05 <sup>a</sup>	13.20	19.80
Adper Single bond 2	FS	12	14.22±5.58 <sup>a</sup>	7.03	29.01
	WS	12	28.92±8.75 <sup>b</sup>	17.80	44.10

### Microtensile bond strength measurement

In order to carry out the  $\mu$ TBS testing, the specimens were prepared as follows: Each prepared molar was sectioned by a diamond disk (Mashhadnamo, Mashhad, Iran) at 300 rpm under water cooled conditions in both vertical and horizontal directions so that the stick specimens including dentin-composite are obtained at an approximate cross-section of 1mm<sup>2</sup>. The cuts direction was in the longitudinal axis of the molar and parallel with the CEJ to obtain the bond strength of the flat floor and the flat wall, respectively. A maximum of 12 sticks were obtained from each molar for each group (Figure 1). Each prepared sticks were mounted on a  $\mu$ TBS jig with cyanoacrylate adhesive and the tensile strength (Mpa) was applied to the dentin-resin bonding zone at a speed of 0.5 mm/min (ISO TR 1145) and recorded until the failure occurred. In order to compare the mean  $\mu$ TBS values of the studied groups, two way and one-way ANOVA and Dunnett's test were used in SPSS ver. 21. P<0.05 was considered as the significant level.

### Determining the failure mode of specimens using stereomicroscope

To determine the failure mode, each specimen was observed under a stereomicroscope (SZ40, Olympus, Tokyo, Japan) with a magnification of 40 x and ranked as follows:

- 1-Adhesive: Composite-bonding or dentin-bonding interface failure.
- 2-Cohesive: Dentin or composite failure
- 3-Mixed: A combination of two above failures.

### Evaluation of the failure morphology by electron microscope (SEM)

From each group, a specimen (a total of 12 specimens for 12 groups) was used to determine the failure surface morphology by the SEM. For this purpose, the selected specimens were placed on an aluminum stub by a conductive adhesive tape (double-sided carbon tape) and, while being placed in a sputtering coating machine (JFC-1100E ION SPUTTER, JEOL, Japan), were coated with the gold-palladium alloy for 10 minutes. Specimens

were analyzed by the SEM device (JEOL JSM-840A, JEOL Ltd, Tokyo, Japan) at magnification rates of 100 x, 500 x and 1000x.

## RESULTS

Table 2 shows the descriptive information of the tested groups. The highest and lowest mean values were obtained for Adper Single Bond 2 adhesive (wall, axial) (28.92±8.75) and Single Bond Universal adhesive (occlusal, floor) (12.83±7.90), respectively.

The Kolmogorov-Smirnov test showed that the data have a normal distribution (P>0.05). The statistical comparison of the means of the studied groups showed a significant difference between the three groups in terms of the types of adhesive as the main factor (P<0.001). Also, the interaction between the adhesive type and the intended wall showed a significant difference (P=0.04). The results of one-way ANOVA (Table 3) showed that the mean  $\mu$ TBS values were not significant in all three adhesives in the (occlusal, floor) orientation, but the mean  $\mu$ TBS values in Adper bonding Single Bond 2 adhesive is significantly more than G-premio Bond and Single Bond Universal adhesives in (axial, wall) orientations (P= 0.05). There was also no significant difference between two types of universal adhesives (P= 0.994). The dentin tubules orientation has a definite effect on the bond strength of Adper single Bond 2 adhesive and increases the bond strength (Table 3).

**Table 3: Post Hoc Tests**

Bonding System	Bonding System	Mean difference	Sig
Single Bond 2	G-premio Bond	13.28500*	.000
	Single Bond Universal	13.67500*	.005
G-premio Bond	Single Bond Universal	.39000	.994

### Microscopic examination

Table 4 shows the results of the stereomicroscope examination of the studied groups (failure mode). As the above table shows, Adper Single Bond 2 had the highest adhesive failure in the axial or wall orientation. However, the results of the failure mode were nearly identical in both universal adhesives in the axial or wall orientation. In the occlusal or floor orientation, the results of the failure mode were similar in all three adhesives.

Table 4: Failure mode

Bonding	Surface	Adhesive	Mix	Cohesive	
				composite	dentine
Adper Single Bond2	Floor	5	6	1	0
	Wall	8	3	1	0
Single Bond Universal	Floor	5	4	2	1
	Wall	4	5	2	1
G-premio Bond	Floor	6	5	1	0
	Wall	5	6	1	0

### The results of SEM analysis of specimens in each group (Fig. 2)

The SEM images of the studied groups are shown in Fig. 2. Images A and D are related to demonstrates Adper Single Bond 2 adhesive, which shows that the smear layer is completely removed and the entrance of the dentin tubules are dilated during the etching process. Images B and E are related to the G-premio Bond adhesive and the images C and F are related to the Single Bond Universal adhesive. As images show, failure occurs at the top of the hybrid layer of self-etch adhesives and under the hybrid layer in Adper Single Bond 2 adhesive (ER).

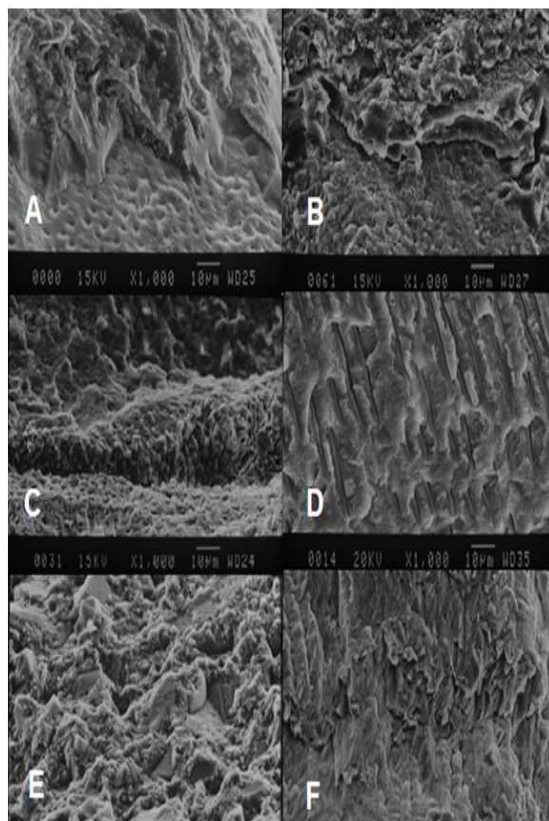


Figure 2: SEM micrographs. A: Floor Adper Single Bond 2; B: Floor GC; C: Floor Single Bond Universal; D: Wall Adper Single Bond 2; E: Wall GC; F: Wall Single Bond Universal

## DISCUSSION

Forming a strong composite resin-dentin bond requires the formation of hybrid layer [20], but the resin-dentin bond strength is independent of hybrid layer thickness [21]. One of the new innovations in dental adhesives is the introduction of universal adhesives. The aim of this study was to determine effect of dentinal tubules orientation on composite-dentin  $\mu$ TBS using universal adhesives. After being used in the one-step self-etch mode, the universal adhesives prevent the collagen collapse by keeping the demineralized dentin moisture. The residual dentin moisture depends on the solvent used in the bonding and clinician's performance according to the manufacturer's instructions [22]. When adhesives are used in self-etch mode, hybrid layer consists of HA debris and the remaining smear layer. Since the total demineralized dentin depth is impregnated with resin monomers, the self-etch adhesives are not technique sensitive and can be easily used in areas where it is difficult to control the sufficient moisture, especially in posterior teeth [22-24]. Therefore, Adper Single Bond Universal and G-premio Universal adhesives were used in self-etch mode in the present study. In this study, the  $\mu$ TBS test was used, which is a valid method for testing the bonding strength. This test is able to more accurately measure tensile strength and allows for an examination of the interfacial bond strength in areas smaller than  $1\text{mm}^2$  [25-28]. It seems that the position and orientation of dentinal tubules can affect the adhesion and adaptation of the composite to the cavity wall [29, 30]. The results of this study revealed that the adhesive type had a significant effect on the  $\mu$ TBS. In other words, etch & rinse mode showed a higher  $\mu$ TBS than the self-etch mode, which is maybe attributed to the thickness of the hybrid layer. The results of Van Meerbeek *et al.*, [31] study, which was conducted on the comparison of SEM and TEM evaluation of resin-dentin bonding region, showed that dentin tubules orientation could have a significant effect on the hybrid layer morphology of etch & rinse adhesives. In other words, when the hybrid layer is thicker and the resin tags are longer in case of the perpendicular (occlusal, floor) dentinal tubules; however, (axial, wall) dentinal tubules orientation leads to the formation of thinner hybrid layer and absence of resin tags. The other finding of this study showed that the dentin tubules orientation had no effect on the preparation  $\mu$ TBS in (occlusal, floor) and

(axial, wall) orientations of self-etch adhesives. The results of this study are consistent with those of Pereira & Phrukkanon's studies. Pereira *et al.*, [32], investigated the effect of internal moisture and differences between different dentin areas on bond strength,  $\mu$ TBS of Clearfil Linear Bond II bond strength in self-etch mode and Bisco in one-step Etch & Rinse mode. Their results showed that  $\mu$ TBS of Clearfil Linear Bond II adhesive didn't differ significantly in different dentinal areas, but the bond strength decreased significantly in the pulp horn region in the one-step Bisco adhesive, and the bond strength was different based on the internal wetness of the region and the adhesive system. The adhesive system should also be selected based on the substrate and the area, in which the bonding occurs. The results of the present study showed that the orientation of the dentinal tubules is not different in the bond strength of the self-etch systems. Also, the results of study by Phrukkanon *et al.*, study [33], which was performed on the effect of dentin position and the tubules orientation on resin-dentin bond strength of Adper Single Bond 2 (ER) and MF-(SE) 102 adhesives, showed that the bond strength was significantly lower in the midroot of Adper Single Bond 2 adhesive; however, there was no significant difference in the position and orientation of dentinal tubules of the MF-102 adhesive. An other important result of the present study was the significant interaction effect of the orientation of dentinal tubules and adhesives used in such way that there was a significant difference between Adper single bond 2 adhesive with Single Bond Universal and Gpremio adhesives in terms of the level in (occlusal, floor) orientation; however, the same  $\mu$ TBS was slightly higher in universal systems, which is probably attributed to the presence of the 10-MDP monomer. Since 10-MDP monomer is not washed in the self-etch mode, the calcium and phosphate molecules, which are obtained from dissolving hydroxyapatite crystals with the 10-MDP monomer, form a chemical bond [34] and create a bond strength level that is higher than etch&rinse systems. However, the HEMA monomer and copolymer of polyalkenoic acid compete with 10-MDP monomer in bonding to the surface of hydroxyapatite crystals and reduce the formation of calcium-10 MDP salts in the dentin-resin interface [35]. For this reason, the lowest bond strength is observed in Single Bond Universal adhesive in perpendicular (occlusal, floor) mode, which is not statistically significant. A statistically significant difference was observed between

Adher Single Bond 2 adhesive with two Universal Bond Universal and Gpremio adhesives in terms of level of  $\mu$ TBS in the parallel (axial, wall) mode in such way that the  $\mu$ TBS of Adper Single Bond 2 adhesive was higher than universal adhesives because the peritubular dentin is thicker in (axial, wall) orientation, and when etch and rinse systems are applied, the phosphoric acid expands the tubule opening and longer resin tags are formed, and in turn leads to increased bond strength. The results of the failure mode also confirmed this finding that higher adhesive failure is observed in Adper Single Bond 2 in the (axial, wall) orientation (Table 4).

In a study on the bonding resin to different dentin surfaces, Sattabanasuk *et al.*, [36] used both Clearfil SE Bond (Se) and OptiBond Solo + (Er) adhesives and the results showed that Clearfil SE Bond had higher shear bond strength in the deep dentin, in the perpendicular to (occlusal, floor) orientation, as compared parallel to the (axial, wall) orientation of the dentin tubules; however, OptiBond Solo + showed higher shear bond strength value in the dentinal tubules in the (axial, wall) orientation, which is consistent with the present study.

Gpremio universal (PH = 1.5) and single bond universal (PH = 2.7) adhesives showed no significant difference in the bond strength in the (occlusal, floor) and (axial, wall) orientations despite having different PH levels, which was inconsistent with the results of Schiltz-Taing's study. In a study on the effect of dentin tubules orientation on the bond strength of self-etch BISCO adhesive with different PHs from 1.1 to 2.7, Schiltz-Taing *et al.*, [37] showed that when the bonding surface was parallel to the (axial, wall) of the dentinal tubules, there is no relationship between shear bond strength and the PH of the self-etch adhesive and when the bonding surface is perpendicular to (occlusal, floor) of the dentinal tubules, decreasing PH levels lead to a reduction in the shear bond strength. Also, this decrease is significant when the PH is lower than 1.8 and there is no significant difference in bond strength at PHs greater than 2.3, which can be attributed to the combination of universal adhesives.

## CONCLUSION

Considering the limitations of this study, the results showed that the of dentin tubules orientation led to no significant differences

between the Gpremio and Single Bond Universal adhesives in terms of the  $\mu$ TBS. Single bond 2 had significantly higher  $\mu$ TBS in (axial, wall) orientation, but showed  $\mu$ TBS value similar to that of universal adhesives in (occlusal, floor) orientation, which was not statistically significant.

### Recommendations

In order to increase the similarity of the results of this study to clinical conditions, it is recommended to design other researches be as clinical studies.

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