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Influence of previous acid etching on dentin bond strength of self-etch adhesives

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Abstract: The purpose of this study was to investigate the effect of previous phosphoric acid etching on the dentin bond strength of single-step self-etch adhesive systems. Facial surfaces of bovine teeth were wet ground with #600 SiC paper. Adhesives were applied with and without previous phosphoric acid etching, and light irradiated. Resin composite was bonded to the adherend surface, followed by light irradiation and storage in water at 37°C. Four groups (n = 10) were made per adhesive system: with and without previous acid etching and with and without thermal cycling between 5°C and 55°C for 10,000 cycles. Specimens were tested in a shear mode at a crosshead speed of 1.0 mm/min. Two-way ANOVA and Tukey HSD test at a level of 0.05 were performed. The changes in dentin bond strengths were different among the adhesive systems tested. In all experimental conditions of this study, the dentin bond strength decreased or remained unchanged with previous acid etching. From the results of this study, previous acid etching might not be acceptable for increasing dentin bond strength of the single-step self-etch adhesive systems. (J Oral Sci 51, 527-534, 2009)

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Introduction

Bonding of composite resins to the dentin substrate has always been based on smear layer pretreatment. Dentin surfaces abraded with abrasive paper or cut with either hand or rotary instruments produce semi-porous structures due to the formation of small particles of the cutting debris, whose thickness, composition, and morphology depend on the instrumentation and dentinal location from which they were created (1). Using different acids, chelation, or oxalate salt solutions, several smear layer treatments, such as total removal, modification, or incorporation of the intact smear layer into the hybrid layer, have been proposed in the previous and current concepts of dentin adhesion (2). While some adhesive systems use the smear layer as a bonding substrate, others employ the "total-etch" technique, which implies the superficial dissolution of the substrate after acid etching. A high-quality hybrid layer requires optimal infiltration of adhesive monomers into the demineralized dentin surface and proper polymerization (3).

Recently, single-step self-etch adhesive systems, which combine the functions of a self-etching primer and a bonding agent, have been developed (2). The use of single-step self-etch adhesives may eliminate technique sensitive factors that negate bonding ability of the restorations (4,5). However, the etching effect of the mild self-etch adhesives

has been reported to be less effective in interacting with a thicker smear layer-covered dentin (6). A question arises whether the creation of a dentin/adhesive interaction zone is sufficient to create a stable adhesion, since the residual smear layer disturbs monomer infiltration into underlying dentin (7,8).

There are studies reporting different conclusions about the bonding effectiveness to dentin of a two-step self-etch adhesive system when placed either with or without previous phosphoric acid etching (9,10). A study which tested the effect of initial phosphoric acid etching on the bond strength of a two-step self-etch adhesive to dentin concluded that the acid etching should be limited to enamel because of impaired dentin bond strengths (9). Another study reported no significant differences among different smear layer treatments with the same adhesive system (10). The potential chemical interaction between the functional monomers and the residual hydroxyapatite depends on each adhesive, in accordance with its composition. This occurs because, as opposed to conventional adhesives that require phosphoric acid etching, the self-etching system demineralizes dentin only partially, leaving hydroxyapatite attached to collagen (11). For single-step self-etch adhesives, little information is available concerning the removal of the smear layer with previous phosphoric acid etching to enhance bonding ability of the adhesives.

Acid etching removes the smear layer and smear plugs, opening dentinal tubules and demineralizing the peri- and intertubular dentin, thus increasing dentin permeability (12). Decalcification depth ranges from 2 to 4 μm and is affected by etchant pH, type, concentration, viscosity, and application time (13). The bonding mechanism of conventional adhesives consists of micromechanical interlocking of resin monomer with the exposed collagen fibrils of wet demineralized dentin. Despite the profound changes acid etching promotes in the chemical composition and physical properties of the dentin matrix, phosphoric acid has been widely used in restorative dentistry as an etching agent for both enamel and dentin (14).

The purpose of this study was to evaluate the effect of previous acid etching on the dentin bond strengths of single-step self-etch adhesives to bovine dentin by means of measurement of shear bond strength. The effect of thermal cycling on the dentin bond strengths of single-step self-etch adhesive systems to dentin was also evaluated. The hypothesis tested was that previous acid etching would increase the bond strength to dentin.

Materials and Methods

The single application self-etch adhesive systems evaluated were Adper Easy Bond (EB; 3M ESPE, St. Paul, MN, USA), BeautiBond (BB; Shofu Inc., Kyoto, Japan), and G-Bond Plus (GP; GC Corp., Tokyo, Japan), as listed in Table 1. All adhesive systems were used in combination with the resin composite Clearfil AP-X (Shade A2, Lot No. 01011A, Kuraray Medical Inc., Tokyo, Japan). Application protocols suggested by each manufacturer are listed in Table 2. A visible light-activating unit Optilux 501 (sds Kerr, Danbry, CT, USA) was used and the power density (800 mW/cm^2) of the light was checked with a dental radiometer (Model 100, sds Kerr) before making specimens.

A total of 120 mandibular incisors extracted from cattle

Table 1 Materials tested

Code	Adhesive system (Manufacturer)	pH	Main components	Lot #
BB	BeautiBond (Shofu Inc.)	2.4	Phosphoric acid monomer, bis-GMA, TEGDMA, water,carboxylic acid monomer, solvent, acetone, initiator	080802
EB	Adper Easy Bond (3M ESPE)	2.7	Methacrylate functionalized polyalkenoic acid HEMA, bis-GMA, methacrylated phosphoric esters, 1,6 hexanediol dimethacrylate, water, ethanol, finely dispersed bonded silica filler, initiator	351008
GP	G-Bond Plus (GC Corp)	1.5	Phosphoric acid ester monomer, 4-MET, dimethacrylate, water, acetone, nano silica filler, initiator	0902041

HEMA: 2-hydroxy ethylmethacrylate,bis-GMA:2,2bis[4-(2-hydroxy-3-methacryloyloxypropoxy)phenyl]propane, TEGDMA: triethyleneglycol dimethacrylate, 4-MET: 4-methacryloyloxyethyl trimellitate

and stored frozen for up to 2 weeks were used as substitute for human teeth (15,16). After removing the roots with a slow-speed saw using a diamond-impregnated disk (Isomet, Buehler Ltd., Lake Bluff, IL, USA), the pulps were removed, and the pulp chamber of each tooth filled with cotton to avoid penetration of the embedding media. The labial surfaces were ground on wet 240-grit silicon carbide (SiC) paper to a flat dentin surface. Each tooth was then mounted in self-curing acrylic resin (Tray Resin II, Shofu Inc.) to expose the flattened area and placed under tap water to reduce the temperature rise from the exothermic polymerization reaction of the acrylic resin. Final finish was accomplished by grinding on wet 600-grit SiC paper. After ultrasonic cleaning with distilled water to remove the excess debris, these surfaces were washed and dried with oil-free compressed air.

A piece of adhesive tape, which had a 4-mm diameter hole, was firmly attached to define the area for bonding. Half of the specimens were phosphoric acid etched (Etchant, 3M ESPE) for 15 s followed by 10 s rinsing with a three-way syringe and air dried. The adhesive was applied on the dentin surface according to the manufacturer's instructions (Table 2). Adhesive applied surfaces were dried with oil-free compressed air and irradiated with the curing unit. A Teflon (Sanplatec Corp, Osaka, Japan) mold, 2.0-mm high and with a 4.0-mm diameter, was used to form and hold the restorative resin on the dentin surface. Resin composite was condensed into the mold and cured for 30 s. The Teflon mold and adhesive tape were removed from the specimen 10 min after light irradiation.

Bonded specimens from each group were divided into two treatment groups of 10 specimens each for testing: Group 1) stored in 37°C distilled water for 24 h after placement, without thermal cycling, and Group 2) stored in 37°C distilled water for 24 h followed by thermal cycling between 5°C and 55°C for 10,000 cycles.

The specimens in each group were tested in shear mode using a knife edge testing apparatus in a universal testing machine (Type 5500, Instron Corp., Canton, MA, USA) at a crosshead speed of 1.0 mm/min. Shear bond strength values in MPa were calculated from the peak load at failure divided by the specimen surface area.

After testing, the specimens were examined under an optical microscope (SZH-131, Olympus Ltd., Tokyo, Japan) at a magnification of ×10 to define the location of the bond failure. The type of failure was determined based on the percentage of substrate-free material as follows: adhesive failure, mixed failure (cohesive failure in composite and adhesive resin with partially adhesive failure), cohesive failure in dentin, and cohesive failure in composite.

A statistical analysis was done to show how the bond strengths were influenced. The data for each group were subjected to ANOVA followed by Tukey HSD test at a level of 0.05. The statistical analysis was carried out with the Sigma Stat software system (Ver. 3.1, SPSS Inc., Chicago, IL, USA).

The treated dentin surface and the fractured surface of the specimens were observed by field emission scanning electron microscopy (SEM). For the treated tooth surface observation, the dentin surfaces were treated and then rinsed with acetone and water to remove the self-etching adhesive. All the SEM specimens were dehydrated in ascending concentrations of *tert*-butanol, and then transferred to a critical-point dryer for 30 min. The surfaces were coated in a vacuum evaporator, Quick Coater Type SC-701 (Sanyu Denshi Inc., Tokyo, Japan), with a thin film of Au. The specimens were observed by SEM (ERA-8800FE, Elionix Ltd., Tokyo, Japan).

Results

The mean shear bond strengths to bovine dentin and failure modes after the test are shown in Tables 3 and 4. For the specimens stored for 24 h in water, the mean bond strengths to bovine dentin ranged from 18.2 to 20.9 MPa without previous acid etching, and ranged from 15.3 to 18.3

Table 2 Application protocols of single-step self-etch systems

Code	Application Protocol
BB	Dispense one drop of liquid into well. Apply to dried dentin for 10 s. Subject to a mild stream of air to dry for 3 s, blow more strongly and light irradiation for 10 s.
EB	Dispense one drop of liquid into well. Apply to dried dentin for 20 s. Subject to a mild stream of air to dry for 5 s and light irradiation for 10 s.
GP	Dispense one drop of liquid into well. Apply dried dentin for 10 s. Subject to a strong stream of air to dry for 5 s and light irradiation for 10 s.

Table 3 Effect of previous acid etching on bond strength (mean (SD) in MPa) to bovine dentin after 24 h storage in distilled water

	Bond strength		Fracture Mode	
	w/o acid etching	w acid etching	w/o acid etching	w acid etching
BB	18.2 (1.3) [c]	17.3 (1.0) [c]	10/ 0/ 0/ 0	6/ 3/ 1/ 0
EB	20.9 (0.4) [a]	15.3 (0.8) [d]	5/ 0/ 4/ 1	4/ 5/ 1/ 0
GP	19.8 (1.8) [a, b]	18.3 (1.4) [b, c]	7/ 1/ 1/ 1	6/ 0/ 4/ 0

Values in parenthesis are standard deviations, $n = 10$
Failure mode: mixed failure/ adhesive failure/ cohesive failure in dentin/ cohesive failure in composite
The values with same superscript letters indicate no statistical difference ($P > 0.05$)

Table 4 Effect of previous acid etching on bond strength (mean (SD) in MPa) to bovine dentin after 10,000 thermal cycles

	Bond strength		Fracture mode	
	w/o acid etching	w acid etching	w/o acid etching	w acid etching
BB	17.1 (1.3) [b]	8.8 (0.6) [d]	10/ 0/ 0/ 0	2/ 8/ 0/ 0
EB	19.1 (1.8) [a]	17.5 (1.9) [b]	3/ 1/ 5/ 1	4/ 4/ 2/ 0
GP	16.7 (1.7) [b]	13.2 (2.4) [c]	8/ 2/ 0/ 0	5/ 5/ 0/ 0

Values in parenthesis are standard deviations, $n = 10$
Failure mode: mixed failure/ adhesive failure/ cohesive failure in dentin/ cohesive failure in composite
The values with same superscript letters indicate no statistical difference ($P > 0.05$)

MPa for the specimens with previous acid etching. When the specimens were subjected to thermal cycling, the mean bond strengths ranged from 16.7 to 19.1 MPa for the specimens without previous acid etching, and from 8.8 to 17.5 MPa for the specimens with acid etching. The changes in dentin bond strengths were different among the adhesive systems tested. From the statistical analysis, there was no significant interaction between previous acid etching and storage conditions. A significant decrease in dentin bond strength was found with previous acid etching for EB, regardless of storage conditions. For BB and GP, no significant differences were found between with and without previous acid etching after 24 h storage in water, but significant decreases in dentin bond strengths were found with previous acid etching after thermal cycling.

The SEM observations of the dentin surfaces after bond strength tests are shown in Figs. 1-3. There were differences in the failure mode among the adhesive systems used. The predominant mode of failure was mixed failure for the specimens made with BB and GP, and there was an increase in adhesive failure for the specimens with previous acid etching (Figs. 1, 3). This tendency was more pronounced for the specimens subjected to thermal cycling. For EB, mixed failure and cohesive failure in dentin were observed for the specimens without previous acid etching, and increase in adhesive failure was observed for the specimens with previous acid etching, regardless of the storage conditions (Fig. 2).

Discussion

Self-etch adhesives are a promising development in adhesive dentistry, especially in terms of reduction of the necessary application steps and the possibility of a chemical interaction with hydroxyapatite-coated collagen fibers (2). However, bonding to dentin with initial phosphoric acid etching still remains critical and has been controversially discussed by various authors (9-11).

When the adherend dentin surfaces were previously treated with phosphoric acid, no changes in bond strength after 24 h were observed for the single-step self-etch adhesives except for EB, which showed significant decrease in bond strength. Thus, the hypothesis that previous acid

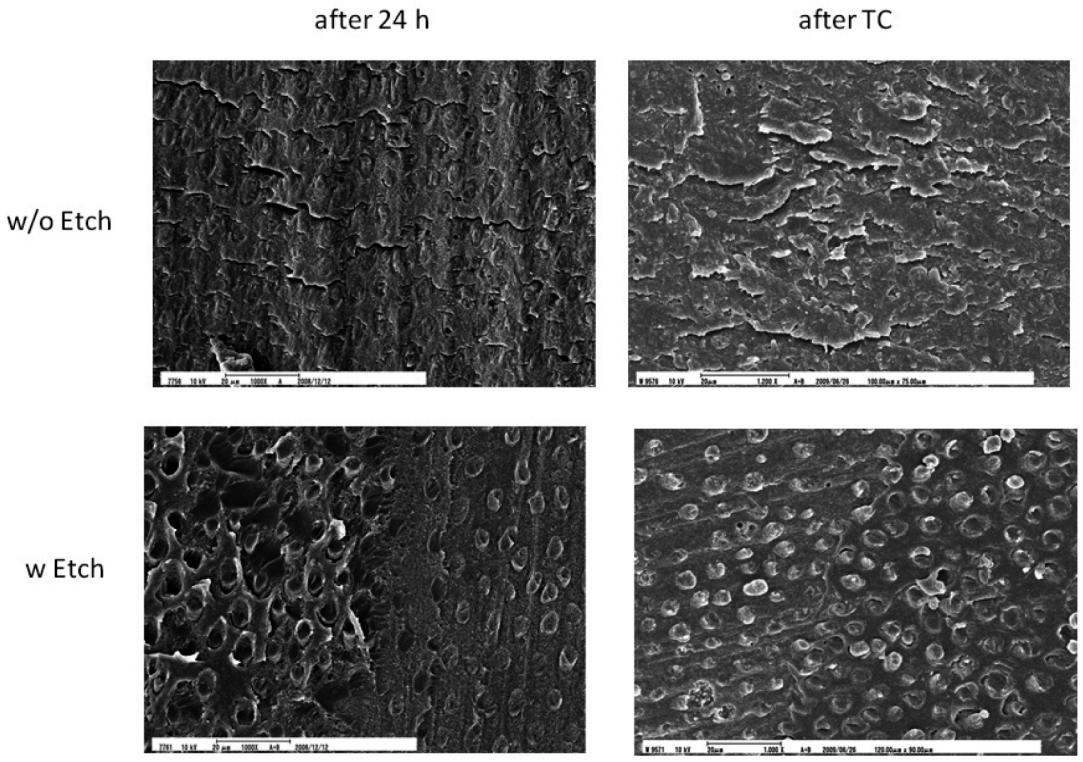

Fig. 1 SEM observation of a fractured dentin surface after bond strength (BeautiBond).

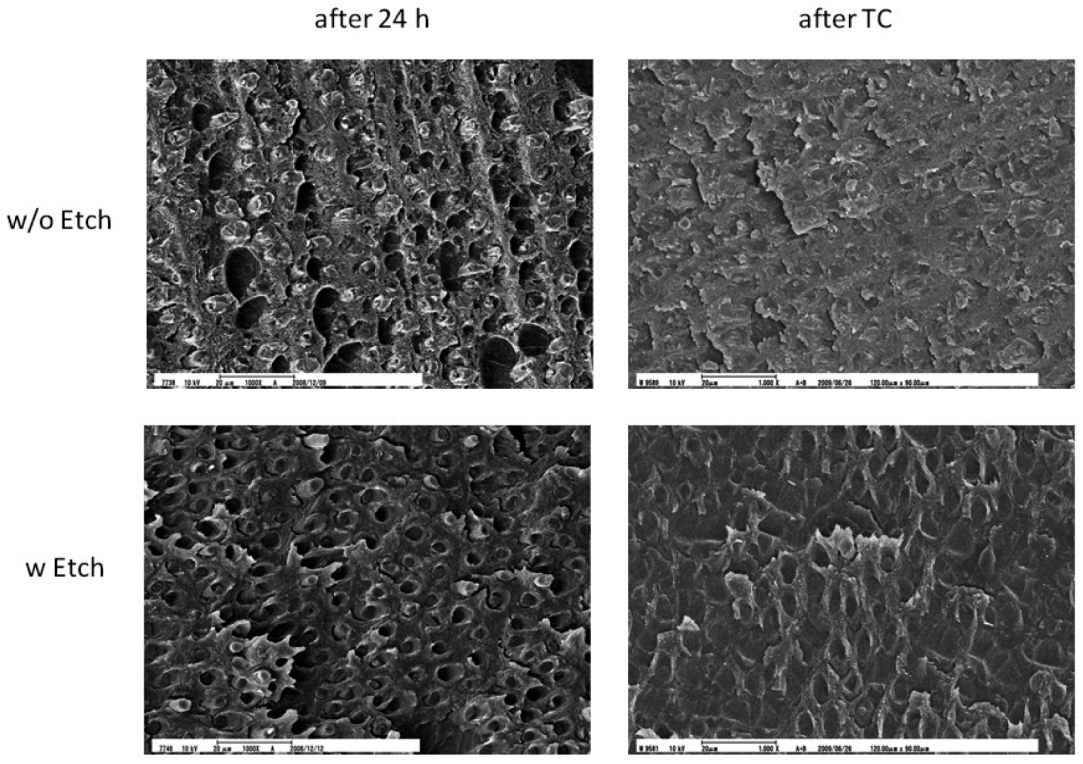

Fig. 2 SEM observation of a fractured dentin surface after bond strength test (Adper Easy Bond).

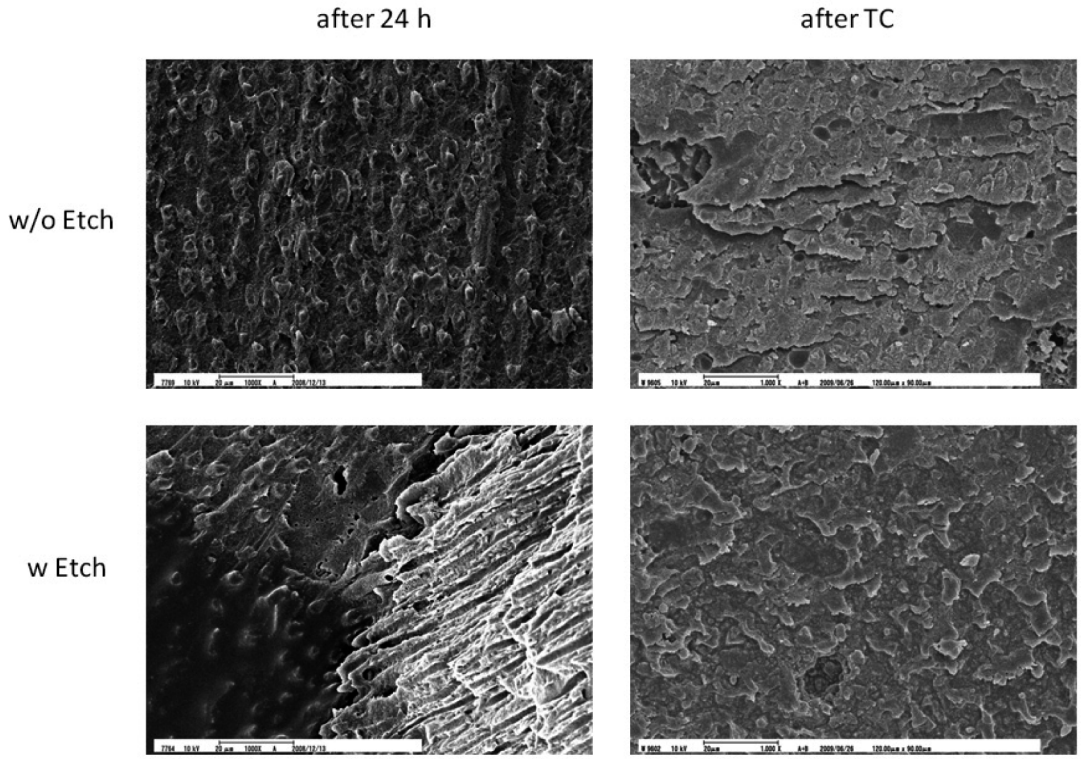

Fig. 3 SEM observation of a fractured dentin surface after bond strength test (G-Bond Plus).

etching would increase dentin bond strengths was rejected. The success of dentin bonding has been believed to be dependent on the infiltration of resin monomers into acid etched dentin followed by polymerization in situ (17,18). It has been indicated that previous enamel etching with 37% phosphoric acid provided statistically significantly higher bond strength values than self-etching adhesive application, and also demonstrated that this procedure decreased the bond strength of resin composite to dentin (9). This has a negative influence on the dentin substrate, which consists of 70% minerals, 20% organic materials and 10% water. When dentin is etched with phosphoric acid, both the extrafibrillar and intrafibrillar mineral are dissolved. Lower bond strength obtained for EB might be explained by incomplete infiltration of the demineralized collagen network by resin monomers (9,19). Collapse of unsupported collagen after phosphoric acid treatment and exposure to air has been shown to inhibit resin monomer penetration to the entire depth of decalcified dentin (20).

On the other hand, no changes in bond strengths were found for BB and GP after 24 h storage in water. Since these single-step self-etch adhesives contain water and low molecular weight hydrophilic monomers, collagen fibrils which had collapsed after phosphoric acid treatment might partially re-expand. Therefore, the hydrophilic components may penetrate into the exposed collagen network. Functional monomers have been shown to interact with Ca^{2+} from apatite crystallites within the partially demineralized hybrid layer to form an insoluble calcium salt that may aid in bonding this resin system to dentin (21,22). Like polyalkenoic acids that can bond chemically to hydroxyapatite or collagen (23), functional monomers have also been shown to bond chemically to both dentine apatite and collagen (24,25). A chemical interaction between hydroxyapatite and functional monomers in the adhesive might contribute to higher bond strengths than the adhesives that rely only on micromechanical retention. The role of collagen fibrils in dentin bonding has not been proven and some reports have revealed that collagen fibrils offer no direct quantitative contribution to the interfacial bond strength (26).

After thermal cycling, significant reductions in bond strength were observed for the adhesives used when the dentin surfaces were treated with phosphoric acid. On the other hand, no significant difference was found in the without previous acid etching group. During the thermal cycling test, the hot water may also accelerate hydrolysis of the resin and extract poorly polymerized resin oligomers (27), leading to a decrease in mechanical properties of the polymers. The complex thermal cycling process offers

many possibilities for entrapment of flaws inside the dentin-resin interface (28). The change in mechanical properties after thermal cycling could result in bond failure trends or tendencies due to weakened adhesive resins, which exist between the dentin and the resin.

The results of this *in vitro* study suggested that there is no benefit of using phosphoric acid prior to application of single-step self-etch adhesives in terms of increasing dentin bond strength. On the other hand, it is impossible from laboratory results to exactly define the clinical lower limit of bond strength that is still able to predict acceptable clinical behavior. Further understanding of the clinical factors that contribute to the bonding characteristics is needed.

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