Effect of fluoride mouthrinse on adhesion to bovine root dentin

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INTRODUCTION

Currently, a growing number of individuals are able to retain their teeth over their lifetime, leading to an increase in the development of root caries in the elderly. However, the challenges involved in the treatment of root caries include moisture control, limited adhesion with dentin being the sole adhesive substrate, and lack of retention in the saucer-shaped root cavities1. Therefore, multiple approaches and aggressive preventive strategies are required to manage these complex circumstances and limit the damage associated with caries, respectively2,3.

It is well known that fluoride ion penetration into the dentin enhances mineralization and increases the acid resistance of the dentin against demineralization4-6. The regular use of fluoride is of great importance in order to prevent and control root caries7,8. Fluoride mouthrinse is an over-the-counter product that is easy to use and allows for higher oral fluoride retention than toothpaste9,10. However, individuals at higher risk of caries might need to use mouthrinses with higher fluoride concentration7,9,11,12.

According to the concept of minimal intervention, intact tooth structures should be preserved during restorations. Non-caries cervical lesions (NCCLs), which are characterized by loss of hard tissue at the cement–enamel junction in the absence of caries, are commonly encountered in dental practice13,14. If the NCCLs advance in depth and width, direct composite restorations, without aggressive surface preparation due to absence of bacterial infection, are required. Nevertheless, there is some controversy regarding the treatment approach of NCCLs15. The dentin surface of the NCCLs can be cleaned with a fluoride-containing polishing paste, as it might have been exposed to fluorides from toothpastes and/or mouthrinses over a long period.

Marginal integration is important for the clinical success of a composite restoration. Ultra-structural examinations of the adhesive–dentin interface after an acid-base challenge have been carried out to investigate the mechanism of secondary caries at the margins of the restorations. Scanning electron microscopic (SEM) and transmission electron microscopic (TEM) observations have revealed the formation of the 'acid-base resistant zone' (ABRZ) was found beneath the hybrid layer (HL) when dentin was treated with a self-etch adhesive system16-18. The morphology of the ABRZ was influenced by the composition of the adhesive systems. It was reported that the release of fluoride from the adhesive created a thicker ABRZ, implying that morphological differences in ABRZ may be an indicator of dentin bond durability19.

In order to estimate the relationship between bonding strength and change in surface property following fluoride use, the chemical state of the fluoride reacted on the dentin surface should be analyzed. X-ray absorption fine structure (XAFS) analysis is one of the most powerful tools used to evaluate the chemical state of the target elements. When the incident X-ray energy becomes equal to the binding energy of the core-level electron (i.e. 'absorption edge'), a steep increase in absorption is noted in the X-ray absorption spectrum. The fine structure

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The purpose of this study was to evaluate the effect of fluoride mouthrinses on dentin bonding performance of a two-step self-etch adhesive; Clearfil SE Bond. Bovine root dentin surface was treated with either 450, 900, or 9,000 ppm F solutions for 30 s (immediate), and continually treated for one month (one month) before the bonding procedures. Microtensile bond strength (µTBS) test and scanning electron microscopic (SEM) observation of the acid-base resistance zone (ABRZ) were performed. Chemical state of fluorine on dentin surface was analyzed by X-ray absorption fine structure (XAFS). The 450 and 900 ppm F fluoride mouthrinses did not influence the µTBS to dentin, while the 9,000 ppm F fluoride solution adversely affected the µTBSs. The fluoride application to dentin significantly enhanced acid resistance at the adhesive/dentin interface including the ABRZ. The XAFS analysis indicated different concentrations of fluoride might create different chemical compounds on the dentin surface, influencing the µTBS results.

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of the spectrum just after the absorption edge reflects the electronic state and the surrounding structure of the target element. The absorption edge energy is an element-specific characteristic. Therefore, the XAFS spectrum provides element-specific information about the target element without the interference of other elements in the biological specimens20,21).

The purpose of this study was to evaluate the effect of different concentrations of fluoride mouthrinses on dentin bonding, using microtensile bond strength (µTBS) test, and ABRZ formation. In addition, XAFS was used to analyze the dentin surface treated with fluoride mouthrinse. The null hypothesis of this study was that application of different concentrations of fluoride mouthrinses does not influence dentin bonding and the adhesive–dentin interfacial morphology.

MATERIALS AND METHODS

Materials

The compositions of the fluoride mouthrinse and an adhesive system used in this study are listed in Table 1. The 450 ppm F (everyday use) and 900 ppm F (once per week use) NaF solutions were prepared by mixing one package (1.8 g) of the mouthrinse kit (MG, MIRANOL Granules 11%, Bee Brand Medico Dental, Osaka, Japan) with 200 and 100 mL distilled water, respectively. The 9,000 ppm F solution was prepared by adding NaF (Wako Pure Chemical, Osaka, Japan) to the 900 ppm F solution in order to adjust the concentration of the solution.

A two-step self-etch adhesive, Clearfil SE Bond (SE, Kuraray Noritake Dental, Tokyo, Japan), was used in this study. A functional monomer, 10-methacryloyloxydecyl dihydrogen phosphate (MDP) was present in the SE primer and the SE bonding agent17,18,22).

µTBS testing

An outline of the µTBS test is schematically presented in Fig. 1. The root of the bovine tooth was perpendicularly divided into two parts using a low-speed diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA) under water cooling. Each piece was embedded in epoxy resin (Epoxicure Resin, Buehler). The root dentin surface was ground with #600-grit SiC paper to produce a standardized smear layer. The specimens were then divided into two main groups according to the storage period. In the immediate groups, the dentin surfaces were treated with 100 µL of 450, 900, or 9,000 ppm F solutions for 30 s, rinsed with distilled water and dried gently, whereas in the one month groups, the dentin surfaces were continually treated with one of the three fluoride mouthrinses for one month before bonding. According to the manufacturer’s instructions, treatment with the 450 ppm F solution was performed for 30 s every day, whereas treatments with the 900 and 9,000 ppm F solutions were conducted for 30 s on a weekly basis. All specimens were stored in artificial saliva (1.0 mM CaCl2, 3.0 mM KH2PO4, 100 mM acetate, 100 mM NaCl, 0.02% NaN3; pH 6.3)3,22-25) at 37°C without the time for the fluoride treatments.

The specimens were then bonded with the SE adhesive according to manufacturer’s instructions. A halogen light curing unit (Optilux 501, Demetrom, Danbury, CT, USA) was used for the light curing of the adhesive. A resin composite (Clearfil AP-X, shade A2, Kuraray Noritake Dental) was placed in two increments (2 mm thick each) and light cured for 40 s each. After 24 h storage in 37°C distilled water, the bonded specimens were perpendicularly sectioned into serial slabs at the resin–dentin interface. Each slab was further sectioned into 1.0×1.0 mm resin–dentin beams. The specimens were then fixed using glue (Model Repair, Dentsply-Sankin, Tochigi, Japan) and stressed in tension at a crosshead speed of 1 mm/min using a universal testing device (EZ-test, Shimadzu, Kyoto, Japan).

Failure mode analysis

After the µTBS test, the debonded specimens of the dentin side were coated with gold sputter and observed under the SEM (JSM-5310LV, JEOL, Tokyo, Japan). The mode of fracture was assessed at nine areas in each specimen. In each area, the failure mode was recorded as one of

Table 1 Materials used in this study

| Brand name       | Code   | Manufacturer       | Composition                                                                 |
|------------------|--------|--------------------|-----------------------------------------------------------------------------|
| Fluoride mouthrinse |       |                    |                                                                             |
| Miranol Granules  | 11%    | Bee Brand          | Sodium Fluoride, D-Mannitol, Xylitol, Macrogel 6000, Sodium dihydrogen Phosphate |
|                  |        | Medico Dental, Osaka, Japan | dihydrate, Cetylpyridinium chloride, Ethyl parahydroxybenzoate, Propyl parahydroxybenzoate, Hydroxypropylcellulose, Cinnamon oil, l-Menthol, Perfume |
| Adhesive system | Clearfil SE Bond | SE | Primer: | Kuraray Primer: MDP, HEMA, DET, Hydrophilic DMA, CQ, Water, (pH 2.0) |
|                  |        |                    | Bond: | Noritake Bond: MDP, Bis-GMA, HEMA, Hydrophobic DMA, DET, Silanated colloidal silica, CQ |
|                  |        |                    | Dental, Tokyo, Japan |                                                                 |

Bis-GMA: bisphenol-A-glycidyl methacrylate; CQ: camphorquinone; DET: N, N-diethanol p-toluidine; DMA: dimethacrylate; HEMA: 2-hydroxyethyl methacrylate; MDP: 10-methacryloyloxydecyl dihydrogen phosphate
the following four categories: complete adhesive failure at the resin–dentin interface (A), complete cohesive failure in the resin composite (R), complete cohesive failure in the dentin (D), and complete cohesive failure in the bonding resin (B). The percentage of each fracture category was calculated based on the frequency of the mode of fracture observed in a total of 270 areas in each experimental group (30 specimens with 9 areas each).

**SEM observation of ABRZ formation**
Sample preparations for SEM observations of ABRZ formation were carried out as described previously (Fig. 2). Bovine root dentin blocks were cut parallel to the tooth axis using a low-speed diamond saw (Isomet, Buehler) and embedded in epoxy resin (Epoxicure Resin, Buehler). The root dentin surfaces were ground with #600-grit SiC paper under running water. The aforementioned methods of fluoride mouthrinse treatments were applied for one day or one month. Subsequently, the dentin surface of each block was treated with the SE adhesive system according to the manufacturer’s instructions. A flowable resin composite (Estelite Flow Quick, Tokuyama Dental, Tokyo, Japan) was then placed between pairs of prepared dentin and light-cured for 40 s from the top and bottom surfaces to create a root dentin sandwich (Fig. 2). After the bonded specimens were stored in distilled water for 24 h, each specimen was sectioned perpendicular to the adhesive–dentin
interface using a diamond saw and embedded in epoxy resin. The bonded specimens were subjected to acid-base challenges based on the previous study6. Each specimen was first stored in 100 mL of a buffered demineralization solution (2.2 mmol/L CaCl2, 2.2 mmol/L NaH2PO4 and 50 mmol/L acetic acid at pH 4.5) for 50 min to create artificial secondary caries. The specimens were immersed in 5% NaClO for 20 min in an attempt to remove any demineralized dentin collagen fibrils and rinsed with running water for 30 s. A 4-META/MMA-TBB resin (Super-Bond C&B, Sun Medical, Moriyama, Japan) was applied without acid-etching of the treated surface in order to prevent the edge of the adhesive from tearing away during polishing. After curing of the 4-META/MMA-TBB resin, the specimens were sectioned perpendicular to the adhesive–dentin interface and polished with diamond pastes (Struers, Copenhagen, Denmark) down to 0.25 µm. The polished surfaces were etched with an argon-ion beam (EIS-IE, Elionix, Tokyo, Japan) directed perpendicular to the polished surface for 30 s with an accelerating voltage of 1 kV and an ion current density of 0.2 mA/cm²; this was done to create sharp etches on the HL. The specimens were then gold-sputter coated, and the morphological changes of the adhesive–dentin interface due to acid-base challenge were observed using a SEM (S-4500, HITACH, Tokyo, Japan) under ×3,500 magnification.

**Image J analysis of the area of ABRZ**

In order to compare the acid-base resistance at the interface quantitatively, the square flame (15×15 µm) was set up on the SEM image as shown in Fig. 3. The left side of the flame was set on the HL at the adhesive interface and the upper side of the flame was placed on the original surface of the bonded specimens. The defined area including the ABRZ and dentin were measured using Image J after SEM observation. B: bonding, CR: composite resin, D: dentin, HL: hybrid layer, OL: outer lesion.

**RESULTS**

**µTBS measurement**

The µTBS values of SE to the dentin are summarized in Table 2. Two-way ANOVA revealed the µTBS values were significantly influenced by the two factors; “dentin treatment” and “storage period” (p<0.05). There was a significant interaction between the two factors (p<0.05). Two-way ANOVA and t-test with Bonferroni correction revealed that the immediate groups yielded significantly higher µTBSs values than the one-month groups in each treatment group (p<0.05). No significant differences in µTBS were noted among the 450 ppm F and 900 ppm
Table 2  μTBS values of SE to dentin (MPa)

| Mouthrinse | Immediate       | One month     |
|------------|-----------------|---------------|
| Control    | 85.5±13.4 a,A   | 69.7±5.6 b    |
| 450 ppm F  | 83.0±11.2 a,B   | 68.0±14.4 b   |
| 900 ppm F  | 83.4±11.3 a,c   | 65.7±9.4 b    |
| 9,000 ppm F| 73.7±9.3 d     | 51.7±8.7      |

Values represent mean±SD, n=30 per group.
Within each column, values indicated by the same lowercase letters are not significantly different.
(Two-way ANOVA and t-test with Bonferroni correction, p>0.05)
Within each rows, values indicated by the same capital letters are not significantly different.
(Two-way ANOVA and t-test with Bonferroni correction, p>0.05)

Table 3  The results of the fracture mode analysis of the debonded area (%)

| Mouthrinse | Immediate A/R/D/B | One month A/R/D/B |
|------------|-------------------|-------------------|
| Control    | 13.2 b/27.2/31.6/30.0 | 3.7 d/38.5/27.4/30.4 |
| 450 ppm F  | 14.8 b/15.4/43.7/26.1 | 22.2 c/15.6/16.1/46.1 |
| 900 ppm F  | 23.0 b,c/8.7/30.0/38.3 | 18.9 d,e/23.0/19.6/38.5 |
| 9,000 ppm F| 56.7 c/10.7/24.4/8.2 c | 25.2 e/27.8/14.8/32.2 |

Values identified with different letters are not significantly different (Mann-Whitney test, p>0.00178).
complete adhesive failure at the resin-dentin interface (A), complete cohesive failure in resin composite (R), complete cohesive failure in dentin (D), complete cohesive failure in bonding resin (B).

F treatment groups and the controls (p>0.05) in both immediate and one-month groups; however, the μTBS values of the 9,000 ppm F groups were significantly lower than that of other groups (p<0.05).

Fracture mode analysis
The results of the fracture mode analysis in the debonded specimens after μTBS testing are summarized in Table 3. Mann-Whitney U test indicated no statistically differences in fracture mode except for the adhesive failure (A) (p>0.00178). In the immediate group, significant differences in mode A were noted between the controls and the 9,000 ppm F groups, and between the 450 ppm F and 9,000 ppm F groups (p<0.00178). Similarly, significant differences in mode B were observed between the 9,000 ppm F group and the other groups (p<0.00178). In the one-month group, significant differences in mode A were seen between the controls and the 450 and 9,000 ppm F groups (p<0.00178).

SEM observations of the adhesive-dentin interface after acid-base challenge
Typical interfacial morphologies between the adhesive and the dentin after acid-base challenges are shown in Figs. 4 and 5. SEM images indicate good bonding between the adhesive and dentin after acid-base challenge. An outer lesion (OL), which was created due to mineral loss following the acid-base challenge, was observed in all the groups. However, the depth of the OL was variable, indicating that the resistance to acid may vary with differences in fluoride application. The HL was hardly visible at the adhesive-dentin interface for each adhesive system. In the immediate group (Fig. 4), thickness of the ABRZ measured at the mid portion of the OL was about 1.5 µm in the control (Fig. 4a). However, the sloped ABRZ formation was predominantly seen in the 450, 900, and 9,000 ppm F groups (Figs. 4b, c and d). As the concentration of fluoride mouthrinse increased, the thickness of the slope appeared to increase. In the one-month group (Fig. 5), the ABRZ was thicker in the control (approximately 2.5 µm, Fig. 5a) than in the immediate group (Fig. 4a). The slope formation of the ABRZ in the mouthrinse groups (Figs. 5b, c and d) was also much thicker than that in the immediate groups (Figs. 4b, c and d).

Changes in the size of the demarcated area inside the flame (15×15 µm) are summarized in Table 4. Two-way ANOVA revealed the areas were significantly influenced by the two factors; “dentin treatment” and “storage period” (p<0.05). However, there was no significant interaction between the two factors (p>0.05). In addition, two-way ANOVA and t-test with Bonferroni correction revealed that the areas in the one month group were significantly larger than in the immediate groups (p<0.05). The areas of the 450, 900 and 9,000 ppm F groups were significantly larger than those of
Fig. 4 SEM images of the adhesive–dentin interface after acid-base challenge in the immediate groups. 

a. control, b. 450 ppm F, c. 900 ppm F and d. 9,000 ppm F (magnification ×3,500). ABRZ: acid-base resistant zone, B: bonding, CR: composite resin, D: dentin, OL: outer lesion.

Fig. 5 SEM images of the adhesive–dentin interface after acid-base challenge in the one month groups. 

a. control, b. 450 ppm F, c. 900 ppm F and d. 9,000 ppm F (magnification ×3,500). ABRZ: acid-base resistant zone, B: bonding, CR: composite resin, D: dentin, OL: outer lesion.

Table 4 The area inside of the flame (15×15 µm²) demarcated (µm²)

| Fluoride concentration | Immediate   | One month  |
|------------------------|-------------|------------|
| Control                | 44.5±9.6    | 72.6±15.8 b,A |
| 450 ppm F              | 69.7±15.2 a | 96.1±18.9 c,B |
| 900 ppm F              | 66.0±12.1 a | 92.5±18.5 c,C |
| 9,000 ppm F            | 75.9±13.7 a | 126.3±14.6 d,D |

Values represent mean±SD, n=20 per group. 
Within each column, values indicated by the same superscript letter are not significantly different. 
(Two-way ANOVA and t-test with Bonferroni correction, p>0.05) 
Within each rows, values indicated by the same capital letters are not significantly different. 
(Two-way ANOVA and t-test with Bonferroni correction, p>0.05)

the controls in the immediate groups; nevertheless, no significant differences were observed among the three treatment groups (p>0.05). In the one month groups, the 9,000 ppm F group showed the largest area among the groups (p<0.05), followed by the 450 and 900 ppm F groups, with no significant differences between them (p>0.05).

Surface analysis with XAFS

Figure 6 shows the normalized F K-edge XAFS spectra of the treated with fluoride contained mouthrinse solutions specimens in the immediate (a) and the two weeks (b). Both FAp and CaF₂ presented with two clear peaks at around 688 and 692 eV, but the peak height balances were different in each of them. In addition, the edge energy, which is the steeply increasing energy on the spectra, was slightly different between CaF₂ and FAp (683 vs 678 eV, respectively). The fluorinated specimens treated with both 450 and 900 ppm F showed similar F K-edge XAFS spectra. Those of the two weeks groups showed more clear spectra compared to those of the immediate groups, because larger amount of F was incorporated in the dentin surface with longer treatment. However entire shape, the edge energy and peak balances of those spectra of both groups were similar to that of FAp but not CaF₂. Therefore, the absorbed fluorine on the surface of those specimens would be formed FAp. In contrast, spectra of specimens treated with 9,000 ppm F with both immediate and two week groups presented with higher edge energy and second peak become unclear compared with the specimens treated with lower fluorine concentrations. Therefore, the chemical state of fluorine in specimens treated with 9,000 ppm F was different from that in the other specimens and rather similar to that of CaF₂.
DISCUSSION

Frequent exposure of fluoride products to dental tissues is beneficial for caries prevention \(^{9,29,30}\). In the present study, dentin specimens were soaked in artificial saliva for one month in order to simulate the frequent use of fluoride mouthrinses at home. The artificial saliva, composed of the mineral components of normal saliva, was expected to accurately represent dentin remineralization \(^{25,26,31}\), however, it was protein-free, because of difficulty of using saliva protein. It was suggested that saliva proteins could be employed to control mineral recovery of the lesion body \(^{29}\).

Clearfil SE Bond is a two-step self-etch adhesive system containing MDP, which is as a functional monomer and has several important roles such as etching of tooth substrates, enhancing monomer penetration, and imparting the adhesives with potential for chemical interactions \(^{22,31-34}\). It was reported that the chemical bonding potential of hydroxyapatite to Ca varied with the types of functional monomers used \(^{34-36}\).

Similar to previous studies, a high bond strength to dentin was obtained in the control group (no treatment) in the present study \(^{5,26}\). In addition, there were no significant differences in µTBS among the control, 450 ppm F, and 900 ppm F in both the immediate and one month groups indicating that regular use of fluoride mouthrinses does not influence dentin bonding performance after using a selfetch adhesive (\(p>0.05\)). However, the µTBS values were significantly decreased in the 9,000 ppm F groups (\(p<0.05\)). Generally, high concentrations of NaF are believed to be effective in increasing the acid resistance of the dentin \(^{8,36,37}\) and possibly in reducing the effect of the self-etching primer. µTBS values in the one month groups were significantly lower than those of the immediate groups, including the controls (without fluoride treatment, \(p<0.05\)). The storage of the tooth in artificial saliva before bonding may have enhanced the mineralization of the smear layer on the ground dentin making it difficult for the self-etching primer to remove the smear layer and penetrate into the underlying dentin. Lowest µTBS values were observed in the 9,000 ppm F group after one month storage in the solution, providing the severest condition for dentin bonding using the self-etching adhesive. Fracture mode analysis of the debonded specimens indicated significantly higher proportions of adhesive failure in the 9,000 ppm F in both the immediate and one month groups, indicating the adverse effects of high concentrations of fluoride on dentin bonding (\(p<0.05\)).

XAFS analysis of bovine dentin powder suggested the formation of FAp-like structures on the dentin surface treated with the 450 and 900 ppm F fluoride mouthrinse solutions in the immediate and two weeks; in contrast, different XAFS spectra, which appeared to be similar to that of CaF\(_2\), were detected in the specimens treated with the 9,000 ppm F solution in both groups. It is well known that application of high-concentration topical fluoride solutions such as acidified phosphate fluoride (APF) and sodium fluoride (NaF) for professional use to sound dentin in hibits the demineralization through the formation of calcium fluoride (CaF\(_2\))-like compounds \(^{38,39}\). These
findings imply that different fluoride concentrations result in the formation of different chemical compounds on the dentin surface and may explain the differences in μTBS values and morphological appearance of the ABRZ in this study.

In a previous study, formation of an ABRZ layer adjacent to the HL at the adhesive–dentin interface after acid-base challenge was noted following the use of a self-etch adhesive. Unfortunately, the HL was hardly visible at the adhesive–dentin interface for each adhesive system under ×3,500 magnification because of the thin layer less than 1 μm thick. The morphology of the ABRZ, which depended on the composition of the adhesive systems, was variable. Previous studies have reported that fluoride-contained in the adhesive influenced the slope formation of ABRZ. Kirihara et al. reported the fluoride concentration had to reach a threshold in order to form the slope. The results of the current study demonstrated that the slope formation of ABRZ was created by the application of fluoride to the dentin surface before bonding with a fluoride-free adhesive. It was reported that MDP adheres to hydroxyapatite readily and intensively, forming a less soluble salt. The protection of the residual apatite crystals in dentin by the functional monomer is a key to prevent wall lesion along the interface. Moreover, the thick, sloped junction formed with the fluoride mouthrinse treatment should serve as the demineralization resistant front.

Quantitative assessment of the acid resistance at the interface was performed using the ROI mode of the digital image analysis software. In the immediate groups, the acid-resistant areas treated with 450, 900, and 9,000 ppm F mouthrines were significantly larger than those without treatment (p<0.05, Table 4). However, there were no differences in the size of the areas among them, indicating that the application of 450 ppm F NaF was sufficient to increase the acid resistance at the interface without adversely affecting the dentin bond strength. The area was significantly larger in the one month groups than in the immediate groups suggesting that repeated treatments of fluoride could enhance the acid resistance of the adhesive–dentin interface, thereby increasing the durability of the bonding (p<0.05). Characterization of fluoride products, such as FAp and CaF₂, created at the interface using the different concentrations of fluoride mouthrines should be characterized. In addition, further study should be carried out to evaluate the effect of different concentrations of fluoride mouthrines on dentin bonding durability.

CONCLUSION
Fluoride mouthrines containing 450 and 900 ppm F NaF did not influence the adhesive–dentin strengths; yet, high concentrations of fluoride demonstrated adverse effects on the μTBS. The resistance of interfacial areas including the ABRZ was significantly enhanced following fluoride application. Application of fluoride on the root dentin resulted in slope formation in the ABRZ. Surface analysis revealed the presence of FAp on the dentin surface after application of the 450 and 900 ppm F mouthrines, while CaF₂-like chemicals were observed following 9,000 ppm F NaF application, thus enhancing dentin bonding performance.

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