Micromorphology analysis and compare bond strength of two adhesives to different degrees of dental fluorosis

Shuangfeng Liu  
Affiliated Hospital of Inner Mongolia Medical College

Yanxia Zhu  
Affiliated Hospital of Inner Mongolia Medical College

Tana Gegen (endotana@163.com)  
Affiliated Hospital of Inner Mongolia Medical College

Research

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Abstract

Objectives: The aim of this study was to evaluate bond strength and morphological analysis of the all-etching bonding system and self-etching bonding system to different degrees of fluorosed enamel.

Methods: Teeth which were indicated for extractions for orthodontic or periodontal problems were selected. The collected teeth were mainly extracted due to orthodontic or periodontal disease. According to the Dean's index and the Thylstrup and Fejerskov (TF) index, 180 extracted teeth were divided into three groups of mild, moderate and severe dental fluorosis, 60 teeth in each group. The teeth in each group were randomly divided into 2 subgroups (n=30) that were subjected to the all-etching bonding system (Prime & Bond NT) and self-etching bonding system (SE-Bond). Each group of adhesives was used to bond Z350 universal resin (3M) to the etched dental enamel. Tensile and shear testing were used to determine the bond strength. After the tensile testing, the fractured specimens were examined under scanning electron microscopy (SEM) and confocal laser scanning microscopic (CLSM).

Results: The Prime & Bond NT had statistically significant on tensile and shear strength for mild fluorosis enamel (P<0.05), but it had no significant difference for moderate and severe dental fluorosis (P>0.05). Self-etching bonding system (SE-Bond) had no statistical significance on the tensile and shear strength for mild, moderate and severe dental fluorosis (P>0.05). SEM and CLSM showed that the mild fluorosis enamel crystals were relatively dense and a small amount of resin remained. The moderate fluorosis enamel crystals were loosely arranged and the gaps were widened. The severe fluorosis enamel crystals were irregularly arranged. The disorder was aggravated, and the dentinal orifice exposed by partial enamel exfoliation can be seen.

Conclusions The bonding strength of mild fluorosis enamel to Prime & Bond NT was better than that of SE-Bond, cohesive failure is the most common failure method in this study. And there is no difference in the bonding strength of SE-Bond to different degrees of DF. Therefore, we suggested that the all-etching adhesive system was used to treat mild fluorosis teeth in the clinic. For moderate to severe fluorosis teeth, we may prolong the application time of self-etching adhesion to enhance enamel-etching pattern and promoted similar results in terms of adhesive enamel bond strength.

Introduction

In 1936, it was shown that the increase of fluoride content in water caused dental fluorosis, which was an alteration of tooth enamel that can be observed as spots ranging from whitish to dark brown color and that, in severe cases, leads to the loss of tooth enamel[1]. In succession, the study on the pathogenesis of DF has been widely studied by scholars, which was found that the exposure of high-fluorine potable water and the absorption of fluoride in food caused dental fluorosis[2]. It was caused by the excessive amount of fluoride in the developmental process of teeth with abnormal surface morphology. DF was also a common and prominent disease in the early stage of chronic fluorosis, and its severe cases could also have skeletal fluorosis, resulting in brittle bones that are susceptible to tensile forces[3].
Fluorosis of the enamel is characterized by a reduced mineral content, resulting in surface and subsurface porosities and subsequent visual, physical changes[4]. Although most developed countries and regions have effectively solved this public health issues and the quality of water has been improved, there are still some developing countries with some DF patients. Clinically, dental fluorosis compromises the tooth's aesthetics, ranging from narrow white lines following the perikymata to discrete white opaque areas, or to an entirely chalky-white tooth surface, depending on the severity[5]. In some patients, the enamel may become so porous that the outer layers break down and the exposed porous subsurface becomes discolored from light to dark brown[6]. The appearance of this discoloration, as well as pitting of the enamel surface, may be aesthetically objectionable to the extent that the patient may experience social repercussions.

Various treatments and managements such as bleaching, microabrasion, composite restorations, veneering, crowning, or a combination of 2 approaches have been proposed to correct the effects of dental fluorosis [5, 7-9]. However, due to the physical and morphological changes induced by dental fluorosis, bonding to this substrate still was a clinical challenge, because fluorapatite was more resistant to acid dissolution than hydroxyapatite[10]. Although good results have been obtained on sound enamel, there is no consensus regarding the application of phosphoric acid to fluorotic enamel[11].

Therefore, the aim of this study was to perform an in vitro evaluation of the bonding strength of the all-etching bond system and self-etching bond system to different degrees of DF by the tensile test and shear test. In addition, stereoscopic microscope and confocal laser scan microscope were used to observe the ultrastructure of the bonding interface. The results will provide clinicians with relevant data to assist to select the most effective adhesive systems for the treatment of DF with different degrees.

Materials And Methods

1. Collection and processing of samples

The study protocol was approved by the Ethics Committee of the Affiliated Hospital, Inner Mongolia Medical. Samples were collected from study subjects who had provided written, informed consent to participate in the study. The 30 samples from the control group were derived from healthy subjects undergoing permanent tooth extraction for orthodontic purposes. The DF teeth were caries free and had been extracted due to periodontal reasons. Exclusion criteria were as follows: 1. The surface had cracks and caries. 2. DF had any treatment. 3. DF had been soaked in hydrogen peroxide. Informed consent was obtained from the subjects before the extraction to use the extracted teeth for the research. The diagnosis of the degree of dental fluorosis was performed by applying Dean's index (DI) [12] and the Thylstrup-Fejerskov Index (TFI, Table 1)[4, 6, 13] [TFI: 0, Healthy enamel; 1-3, mild; 4-5, moderate; 6-9, severe fluorosis]. 180 extracted DF teeth were divided into three groups of mild, moderate and severe dental fluorosis, 60 teeth in each group. The teeth in each group were randomly divided into 2 subgroups (n=30) that were subjected to the all-etching bonding system (Prime & Bond NT) and self-etching bonding system (SE-Bond). Before being prepared, teeth were washed under running water, and the soft tissue was
removed from the tooth surface with the surgical blade. All the treated teeth were immersed in 1% Chloramine and stored in a 4 °C refrigerator for later use. (Fig 1.)

2. Preparation of experimental specimens

The teeth were cleansed from any debris before using in vitro. The superanhydrite mixed according to a certain proportion was filled in the prepared mould and embedded in the root of dental fluorosis in vitro. At the same time, the crown above the boundary of the cementum was exposed. According to the designed grouping, the enamel surface of mild, moderate and severe fluorosis teeth was used to remove 0.5mm, and the polished interface was observed by stereoscopic microscope (32 ×) to ensure that the remaining tissue surface was still located in the enamel layer. The rectangular pores of 2mm × 3mm were made on one side of the tape with a hole punch, and the porous tape was pasted into the enamel grinding area.

Then, it was respectively used to bond with all-etching bonding system(Prime & Bond NT) and self-etching bonding system(SE-Bond) according to the manufacturer's recommendations(Details of the materials used are summarized in Table 2). Each group of adhesives was used to bond Z350 universal resin (3M) to the etched dental enamel. The composite resin (Z350) was filled in layers under pressure and cured, and finally the resin block with an area of $2 \times 3 \times 2mm^3$ was formed. The prepared experimental specimens were stored in a thermostatic water bath for 24 hours.

3. Mechanical property test

The prepared experimental specimens were fixed on the Universal Capability Tester (WDW-100, China) with a fixture, whose load head was aligned with the bonding surface. And the loading force was continued at a horizontal tensile loading speed of 1.0 mm / min until the test specimen bonding surface was broken. Mean bond strengths were recorded from the maximum load at failure and converted to MPa. The actual bonding interface area was measured by vernier caliper. (Fig 2-4)

The tensile strength and shear strength (MPa) = Maximum breaking load (N) / Bonding area (mm$^2$)

4. SEM to observe bonding surface

After the mechanical property test specimen has been completely dried, it had been immersed in the 1 g/L rhodamine B fluorescent dye solution at 37 °C for 24 hours. After rinsing with distilled water for 10s, the teeth are cut sagittal from the buccal side to the lingual side along the long axis of the tooth through the center of the specimens. The fracture surface of the test specimen was used to observe by Scanning electron microscope (Hitachi S-4800, magnification 5-100000×).To examine the fracture modes of the debonded specimens after the TBS testing, the fractured specimens were observed under a SEM. Fracture mode was classified into four types— Type 1: A/M – adhesive/mixed; Type 2: CE – cohesive-enamel; Type 3: CR – cohesive-resin; Type 4: PF – premature failure.

5. Confocal laser scanning microscopic (CLSM) observation
The samples were rinsed in distilled water and observed using a CLSM (LEXT OLS4100, magnification 500 x) at a relative humidity of 100%. The CLSM used in this study was a video rate instrument, achieving a frame time of 33 ms by the use of an accousto-optic deflector for high speed scanning in one axis. This allows rapid three-dimensional assessments of samples without damage from prolong drying. The utility of this technique for imaging the depth and the shape of lesions is well demonstrated elsewhere[14]. We evaluated the depth of the different degrees of fluorosed enamel from the surface of the undemineralized acid resistant varnish covered tooth part to the deepest demineralized front.

5. **Statistical analysis**

Data are expressed as mean ± standard deviation. Statistical analysis was performed using SPSS software (version 17.0, IBM Corp., Armonk, NY, USA). The bond strengths were compared using enamel type as a fixed factor and bonding approach as a random factor using two-way ANOVA under general linear model. The Pearson was used to explore the association between the failure modes and types of fluorosis for the each bonding approach. The failure modes were put into four groups before analysis; adhesive/mixed (A/M), cohesive-enamel (CE), cohesive-resin (CR) and premature failure (PF). $P<0.05$ was considered statistically significance.

**Results**

1. **Tensile bonding strength (TBS)**

The all-etching adhesive had significant differences in the tensile bonding strength of the mild, moderate and severe dental fluorosis ($P<0.05$). The self-etching adhesive had no significant difference in the tensile strength of the mild, moderate and severe dental fluorosis ($P>0.05$). By comparing the tensile strength of the same degree of dental fluorosis with different adhesives, it can be seen that the tensile strength of the mild dental fluorosis with all-etching adhesive was significantly higher than that of the self-etching adhesive ($P<0.05$) and that moderate and severe dental fluorosis with all-etching adhesive and self-etching adhesive had no significant difference ($P>0.05$). (Table 3, Fig 5)

2. **Shear bonding strength**

All-etching adhesive had significant differences in the shear bonding strength of the mild, moderate and severe dental fluorosis ($P<0.05$). The self-etching adhesive had no significant difference in the shear strength of the mild, moderate and severe dental fluorosis ($P>0.05$). By comparing the shear strength of the same degree of dental fluorosis with different adhesives, it can be seen that the shear bonding strength of the mild dental fluorosis with all-etching adhesive was significantly higher than that of the self-etching adhesive ($P<0.05$), and that moderate and severe dental fluorosis with all-etching adhesive and self-etching adhesive had no significant difference ($P>0.05$). (Table 4, Fig 6)

3. **Observing the fracture interface under the SEM**
The SEM results of the study was found that the enamel of mild dental fluorosis was relatively uniform and dense. A small number of crystals were disorderly arranged, and the interspace of the crystals was slightly widened, and various amounts of adhesive residue were visible. It showed that the fracture occurred between the resin and enamel surface under the influence of force, which was the interface fracture. The bonding strength of mild dental fluorosis was determined by the adhesive. The enamel on the surface of severe fluorosis was severely exfoliated, and even some of the dentin tubules were exposed. The enamel was relatively loose and non-uniform. (Fig7, 8) The fracture forms of the two adhesives are mainly cohesive-enamel, and there is no statistical difference between the two groups (t = 1.660, P > 0.05). (Table 5)

It showed that the enamel strength of severe dental fluorosis was low, and the enamel was exfoliated occurs under the influence of force, which was enamel fracture. Thus, the bonding strength of severe dental fluorosis was determined by the strength of enamel. Moderate dental fluorosis was similar to severe fluorosis, and the enamel was also exfoliated under force, but the degree of enamel exfoliation was lighter than that of severe fluorosis. The electron microscope results were basically consistent with the results of mechanics property test.

4. Observing the fracture interface under the CLSM

The results of the study was found that with the increase of the degree of dental fluorosis, the enamel crystal was loosely arranged. Moreover, the bonding interface after all-etching adhesive bonding process was rougher than the self-etching adhesive, and the bonding interface appeared more uneven. (Fig 9, 10)

Figure legend

Fig.1 Preparation of experimental specimens. A Severe dental fluorosis. B Moderate dental fluorosis. C Mild dental fluorosis. D Normal healthy teeth.

Fig.2 Schematic diagram of TBS and SBS test.

Fig.3 Tensile experiment. A Tensile device. B Fixture. C Bonding interface.

Fig.4 Shear experiment. A Shear device. B Fixture. C Bonding interface.

Fig.5 Histogram analysis of tensile strength determination.

Fig.6 Histogram analysis of shear strength determination.

Fig.7 SEM results of mild, moderate, and severe dental fluorosis fracture interface after all-etching adhesive strength test. Original magnification, 5.00k×; scale bar=10.0μm. (a) Fracture interface of mild fluorosis. There is more adhesive residue in the bond interface. (b) Fracture interface of moderate fluorosis. A small amount of adhesive remained and part of the dentin tubules were exposed. (c) Fracture interface of severe fluorosis. The enamel was completely exfoliated. The dentin tubules were completely exposed, and the crystals were disordered. In addition, the interspace between the crystals was
significantly widened, and the number of enamel columns was small. (d) Normal healthy enamel bonding group as a control group

Fig.8 SEM results of mild, moderate, and severe dental fluorosis fracture interface after self-etching adhesive strength test. Original magnification, 5.00k×; scale bar=10.0μm. (a) Fracture interface of mild fluorosis. The enamel of the bonding surface was relatively uniform and dense. A small number of crystals were disorderly arranged, and the interspace between the crystals was slightly widened, and a small amount of adhesive remains. (b) Fracture interface of moderate fluorosis. The enamel was exfoliated under tension, which was lighter than severe fluorosis. (c) Fracture interface of severe fluorosis. The surface enamel was severely exfoliated, and even some dentin tubules were exposed. (d) Normal healthy enamel bonding group as a control group

Fig.9 CLSM results of mild, moderate, and severe dental fluorosis fracture interface after all-etching adhesive strength test. Original magnification, 100×; scale bar=20μm.

(a) Fracture interface of mild fluorosis. (b) Fracture interface of moderate fluorosis. (c) Fracture interface of severe fluorosis. (d) Normal healthy enamel bonding group as a control group

Fig.10 CLSM results of mild, moderate, and severe dental fluorosis fracture interface after self-etching adhesive strength test. Original magnification, 100×; scale bar=20μm. (a) Fracture interface of mild fluorosis. (b) Fracture interface of moderate fluorosis. (c) Fracture interface of severe fluorosis. (d) Normal healthy enamel bonding group as a control group.

**Discussion**

Fluoride is needed for the normal development of bone and teeth; in high levels, it affects developing teeth and bone. Dental fluorosis (DF) was caused by ingestion of excess fluoride mainly through drinking water[15, 16]. In addition, the use of fluorides has also related to an increase in dental fluorosis. Water fluorination and the use of fluoride such as fluoride toothpaste, specific food / beverages and fluoride supplements are considered to be risk factors for dental fluorosis[1].

In the fluorosed teeth, the highly mineralized enamel surface layer is composed of a mixture of many large and extremely small crystals, and the hypomineralised subsurface area is composed of fairly sparsely arranged large crystals with a few small crystals. Some of the crystals in the subsurface hypomineralised layer exhibit defects such as perforations[17]. In more severe cases, the hypomineralization is so extensive and the outer well mineralized surface layer is rather brittle and chewing forces may result in formation of surface enamel defects appearing as single pits along the perikymata, or the surface enamel is chipped away, corresponding to the incisal edges or cuspal tips. In particular, occlusal surfaces are rapidly worn often to such an extent that the hypomineralized porous layer is abraded away[18].
At present, regarding the theory of dental enamel bonding, some scholars believed that mechanical chimera, physical adsorption and chemical bonding were the main ways of dental enamel bonding[19]. The retention force between the adhesive and the enamel mainly depended on mechanical fitting, so the surface morphology of the enamel would have a certain effect on the bonding strength. Most studies on the adhesive strength of teeth were based on healthy teeth, but the enamel of DF was different from the enamel crystal structure of normal teeth[15]. This study showed that the adhesive and dental enamel were mainly held firmly by the mechanical fitting of the micropores that were produced by the enamel acid etching under the CLSM, so the relationship between the physical state of the enamel surface and the bond strength after acid etching was also very important.

Observing the fracture interface through SEM, we can see that for mild fluorosed enamel, a distinct dissolution of the outer surface was clearly seen with many surface irregularities in terms of shallow fissures and other defects. For moderately and severely fluorinated enamels, the loss of the external microscopic surface was visible and some dentin tubules are exposed. This was consistent with the research results of some scholars. Although fluorapatite may be more acid resistant, other structural changes, such as fluorotic enamel’s outer hypermineralized layer with a porous hypomineralized subsurface and the low surface energy, impair surface wetting. This results in a decrease in the bond strength[20]. In addition, when observing the fracture interface under SEM, cohesive failure is the most common failure method in this study. When the fractured specimens were observed by confocal laser microscope, cohesive failure was the most prevalent mode of failure in moderate and severely fluorosed teeth with previous phosphoric acid etching. This should be taken into consideration as the self-etching primer adhesive system produces a predominant adhesive failure[21]. At the same time, some scholars proved that most of the specimens (98%) exhibited adhesive/mixed failures, 1% showed premature failure, and 1% displayed cohesive failure exclusively within the enamel, or cohesive failure exclusively within the resin composite in DF[6]. When sound and fluorotic enamel are compared, lower bond strengths are obtained to fluorotic enamel, independent of the application technique. These results should be examined further, because fluorotic enamel is more resistant to acidic dissolution due to the presence of fluorapatite, which adversely affects the performance of the adhesive[10]. Some researchers[22] believed that the enamel of DF to produce micropores was affected by acid etching and decalcification. With increasing severity of fluorosis, the subsurface enamel all along the tooth becomes increasingly porous (hypomineralized), and the lesion extends toward the inner enamel[23]. At the same time, from the viewpoint of mechanical bonding[22], it was inferred that if the glazed column of the glazed surface after acid treatment was perpendicular to the bonding surface, it could produce an ideal honeycomb structure with the largest adhesion, and if the partial glaze column after acid treatment was parallel to the bonding surface, it could produce the smallest adhesion. It was further confirmed that the abnormal enamel morphology, whether it was the collapse of the enamel development or the disorder of the glaze column, it would affect the bonding strength. It has been reported that the enamel surface of fluorosed teeth appeared highly uneven and rough showing cracks and fissures. The enamel surface showed pits of varying dimensions in the discolored area of the teeth and these appear as punched lesions on the enamel surface, thus exposing the underlying porous enamel[24]. A recent study on ground sections of
fluorosed teeth reported crescent-shaped hypomineralized areas in enamel and increased interglobular dentin spaces[25].

This study prove that the all-etching bonding system for mild fluorosis teeth has higher bonding strength than the self-etching bonding system in the tensile test and shear test, which is consistent with the research results of many scholars. Due to the special enamel structure of the DF, the micromechanical fitting after acid etching was not good, which affected the bonding effect between the adhesive and enamel[26, 27]. Self-etching adhesives systems use weak acidic monomers to condition the enamel/dentin substrate totally. Some scholars[21] have demonstrated that K-etchent gel (37% phosphoric acid) produced typical etching patterns consistent to phosphoric acid regardless of severity of fluorosis. Etching pattern observed by self-etching primer was shallower to K-etchent, which use MDP as the etching agent. These may have resulted by a shift in the crystal orientation or by enlargement of laminar pores which were reported to be more important diffusion pathways than interprismatic enamel during the acid attack[28]. Many scholars also have discovered that the two-step etch-and-rinse adhesive system in the current study, Single-Bond, uses 35% phosphoric acid with a pH of approximately 0.6. And 35% phosphoric acid was strong acid. The two-step self-etch adhesive SE Bond is classified as a “mild” self-etch with a pH of 2.0. Since self-etch adhesives are less acidic than phosphoric acid, they do not demineralize enamel to the same extent, yielding a less micro-retentive surface and, consequently, a lower bonding strength[29]. In addition, shallow fissures with other irregularities were observed in mild fluorosed enamel[30].

The all-acid etching bonding system used phosphoric acid to etch the tooth enamel before demineralization, and removed the stained layer by acid etching. Compared with the self-etching bonding system, the all acid etching bonding could form a wider penetration area on the surface of the dental fluorosis, and the resin protrusions formed were more uniform, which improved the effect of micromechanical fitting[26, 31, 32]. However, some scholars believed that self-etching bonding systems were inferior to all-etching bonding systems when bonding is performed on moderate and severe fluorosed enamel, which is inconsistent with our results[11, 33]. More detailed examination of the fluorotic enamel results revealed that although fluorotic enamel was more acid resistant, the prolonged application mode improved demineralization, observable as an increase of micro-irregularities and porosities of the prisms in the microscopic analysis. Further, mode also improved the interaction of acidic monomers with prismatic and interprismatic areas[6]. However, there is no significant difference between the all etching bond system and the self-etching bond system of moderate and severe fluorosis. It may be because the degree of demineralization of enamel in moderate and severe fluorosis was low, and the enamel is more fragile and easy to break[30].

Some scholars also believed that the prolonged application time of universal adhesives in SE mode in fluorotic enamel enhanced enamel-etching pattern and promoted similar results in terms of adhesive enamel bond strength[6].

**Conclusions**
The bonding strength of mild fluorosis enamel to Prime & Bond NT was better than that of SE-Bond, cohesive failure is the most common failure method in this study. And there is no difference in the bonding strength of SE-Bond to different degrees of DF. Therefore, we suggested that the all -etching adhesive system was used to treat mild fluorosis teeth in the clinic. For moderate to severe fluorosis teeth, we may prolong the application time of self-etching adhesion to enhance enamel-etching pattern and promoted similar results in terms of adhesive enamel bond strength.

**Declarations**

**Availability of data and materials**

The data supporting the findings are presented in this manuscript.

**Competing interests**

The authors declare that they have no competing interests.

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**Authors' contributions**

Tana Gegen designed the experiments. Shuangfeng Liu and Yanxia Zhu are the co-first author, performing the research, analyzed the data and wrote the paper, which was revised by Tana Gegen. All authors approved the final version of the manuscript.

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**Authors' information**

**First author:**

Shuangfeng Liu, Hohhot Stomatological Hospital, the department of endodontics, Hohhot, 010020, China.

Affiliated Hospital of Inner Mongolia Medical University, the department of stomatology, Hohhot, 010000, China.

Email:1042398988@qq.com

**Co-first author:**
Yanxia Zhu, Affiliated Hospital of Inner Mongolia Medical University, the department of stomatology, Hohhot, 010000, China.

Email: endozhuyanxia@163.com

*Corresponding author* Tana Gegen, Affiliated Hospital of Inner Mongolia Medical University, the department of stomatology, Hohhot, 010000, China

Email: endotana@163.com

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### Tables

**Table 1.** Comparison of tensile strength of dental fluorosis with all-etching adhesive.

(MPa, mean ± standard deviation, n=30)

| Groups                  | Tensile Strength (MPa) | $F$    | $P$   |
|-------------------------|------------------------|--------|-------|
| Mild dental fluorosis   | 7.82±4.20              | 4.610  | 0.014 |
| Moderate dental fluorosis | 4.79±2.69$^a$         |        |       |
| Severe dental fluorosis | 5.04±2.81$^a$         |        |       |

According to the SNK-$q$ method, the difference of $^aP<0.05$ was statistically significant.

**Table 2.** Comparison of tensile strength of dental fluorosis with self-etching adhesive.

(MPa, mean ± standard deviation, n=30)
Table 3. Comparison of tensile strength of different adhesives with the same degree of dental fluorosis. (MPa, mean ± standard deviation, n=30)

| Groups                        | Tensile Strength [MPa] | F    | P  |
|-------------------------------|------------------------|------|----|
| Mild dental fluorosis         | 5.39±3.24              | 0.393| 0.667 |
| Moderate dental fluorosis     | 4.93±2.81              |      |    |
| Severe dental fluorosis       | 4.55±2.11              |      |    |

Table 4. Comparison of shear strength of dental fluorosis with all-etching adhesive. (MPa, mean ± standard deviation, n=30)

| Groups                        | Shear Strength [MPa] | F    | P        |
|-------------------------------|----------------------|------|---------|
| Mild dental fluorosis         | 27.44±2.37           | 27.201| 0.015   |
| Moderate dental fluorosis     | 24.71±2.08\textsuperscript{b} |      |         |
| Severe dental fluorosis       | 23.66±1.64\textsuperscript{b} |      |         |

According to the SNK-\(q\) method, the difference of \(b P < 0.05\) was statistically significant.
Table 5. Comparison of shear strength of dental fluorosis with self-etching adhesive.

(MPa, mean ± standard deviation, n=30)

| Groups               | Tensile Strength (MPa) |  |  |
|----------------------|------------------------|---|---|
| Mild dental fluorosis| 23.94±1.99             | 2.299 | 0.106 |
| Moderate dental fluorosis | 23.81±1.65         | -0.163 | 0.071 |
| Severe dental fluorosis | 22.87±2.56           | 0.520 | 0.163 |

Table 6. Comparison of shear strength of different adhesives with the same degree of dental fluorosis. (MPa, mean ± standard deviation, n=30)

| Groups               | All-etching adhesive MPa | Self-etching adhesive MPa | t value | P value |
|----------------------|----------------------------|----------------------------|---------|---------|
| Mild dental fluorosis| 27.44±2.37                 | 23.94±1.99                 | 6.354   | 0.000   |
| Moderate dental fluorosis | 24.71±2.08             | 23.81±1.65                 | -0.163  | 0.071   |
| Severe dental fluorosis | 23.66±1.64             | 22.87±2.56                 | 0.520   | 0.163   |

Figures
Figure 1

Preparation of experimental specimens. A Severe dental fluorosis. B Moderate dental fluorosis. C Mild dental fluorosis. D Normal healthy teeth.
Figure 2

Schematic diagram of TBS and SBS test.

Figure 3

Tensile experiment. A Tensile device. B Fixture. C Bonding interface.
Figure 4

Shear experiment. A Shear device. B Fixture. C Bonding interface.
Figure 5

Histogram analysis of tensile strength determination.
Figure 6

Histogram analysis of shear strength determination.
Figure 7

SEM results of mild, moderate, and severe dental fluorosis fracture interface after all-etching adhesive strength test. Original magnification, 5.00k×; scale bar=10.0μm. (a) Fracture interface of mild fluorosis. There is more adhesive residue in the bond interface. (b) Fracture interface of moderate fluorosis. A small amount of adhesive remained and part of the dentin tubules were exposed. (c) Fracture interface of severe fluorosis. The enamel was completely exfoliated. The dentin tubules were completely exposed, and the crystals were disordered. In addition, the interspace between the crystals was significantly widened, and the number of enamel columns was small. (d) Normal healthy enamel bonding group as a control group
Figure 8

SEM results of mild, moderate, and severe dental fluorosis fracture interface after self-etching adhesive strength test. Original magnification, 5.00kx; scale bar=10.0μm. (a) Fracture interface of mild fluorosis. The enamel of the bonding surface was relatively uniform and dense. A small number of crystals were disorderly arranged, and the interspace between the crystals was slightly widened, and a small amount of adhesive remains. (b) Fracture interface of moderate fluorosis. The enamel was exfoliated under tension, which was lighter than severe fluorosis. (c) Fracture interface of severe fluorosis. The surface enamel was severely exfoliated, and even some dentin tubules were exposed. (d) Normal healthy enamel bonding group as a control group
Figure 9

CLSM results of mild, moderate, and severe dental fluorosis fracture interface after all-etching adhesive strength test. Original magnification, 100×; scale bar=20μm. (a) Fracture interface of mild fluorosis. (b) Fracture interface of moderate fluorosis. (c) Fracture interface of severe fluorosis. (d) Normal healthy enamel bonding group as a control group
Figure 10

CLSM results of mild, moderate, and severe dental fluorosis fracture interface after self-etching adhesive strength test. Original magnification, 100×; scale bar=20μm. (a) Fracture interface of mild fluorosis. (b) Fracture interface of moderate fluorosis. (c) Fracture interface of severe fluorosis. (d) Normal healthy enamel bonding group as a control group.

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