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

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Abstract

Objectives: To evaluate the effects of two adhesives on the bond strength of different degrees of dental fluorosis (DF).

Methods: Teeth which were indicated for extractions for orthodontic or periodontal problems were selected. 180 extracted teeth were selected with varying degrees of DF based on modified Dean's fluorosis index, including 60 teeth with mild fluorosis, 60 teeth with moderate fluorosis, and 60 teeth with severe fluorosis. 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 laser scanning confocal microscope. (LSCM).

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 LSCM 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. And there is no difference in the bonding strength

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of SE-Bond to different degrees of DF.

Keywords: dental fluorosis, enamel, tensile bond strength, shear bond strength，LSCM

Introduction

Dental fluorosis (DF) was prevalent in many countries that had become a public issue, and it has also increased the burden of worldwide disease, particularly in developing countries, such as China[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 fluorine 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. [3]

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. 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. [4] At present, there are few reports on the bonding strength to different degree of DF.

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] The success of restoration was based on the high adhesive property of the material. 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 for different degrees of DF by tensile bonding strength and shear bonding strength tests. 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 samples from the control group were derived from healthy subjects undergoing permanent tooth extraction for orthodontic purposes. Inclusion criteria were that DF have intact dental tissue. 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. 180 extracted teeth were selected with varying degrees of DF based on modified Dean's fluorosis index, including 60 teeth with mild fluorosis, 60 teeth with moderate fluorosis, and 60 teeth with severe fluorosis. 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). All the collected teeth were immersed in 1% chloramine solution and stored in a 4 °C refrigerator for later use.

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 mold 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). 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 $2\times3\times2mm^3$ 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. The maximum tensile and shear load were recorded when they were fallen off the enamel surface. The actual bonding interface area was measured by vernier caliper.

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

4. Laser scanning confocal microscope to observe fracture 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×) and laser scanning confocal microscope (LEXT OLS4100 ,magnification 500 ×) at a relative humidity of 100%.

The fracture mode was divided into: 1) Fracture mode in resin layer. 2) Fracture mode in enamel layer. 3) Mixed fracture mode of resin-enamel bonding interface.

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). ANOVA and Two independent sample *T* test were used to examine tensile strength and shear bonding strength of each group. $P<0.05$ was considered statistically significance.

Results

1. Tensile bonding strength

The all-etching adhesive had significant differences in the tensile bonding strength of the mild, moderate and severe dental fluorosis ($P<0.05$) in Table 1.The self-etching adhesive had no significant difference in the tensile strength of the mild, moderate and severe dental fluorosis ($P>0.05$) in Table 2. 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 ($P>0.05$) in Table 3.

2. Shear bonding strength

All-etching adhesive had significant differences in the shear bonding strength of the mild, moderate and severe dental fluorosis ($P<0.05$) in Table 4.The self-etching adhesive had no significant difference in the shear strength of the mild, moderate and severe dental fluorosis ($P>0.05$) in Table 5. 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 ($P>0.05$) in Table 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 occured 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.

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 LSCM

The results of the study was found that with the increase of the degree of dental fluorosis, the enamel crystal was loosely arranged. (Fig 3, 4)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 3, 4)

Figure legend：

Fig.1 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.

Fig.2 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.

Fig.3 LSCM 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.

Fig.4 LSCM 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.

Discussion

Fluoride is needed for the normal development of bone and teeth; in high levels, it affects developing teeth and bone. Dental fluorosis (DF) is caused by ingestion of excess fluoride mainly through drinking water. [4, 6]

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.[7] 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. Some researchers[8] believed that the fluoride concentration increased during the period of development and mineralization of DF, leading to enamel dysplasia, which disrupted the enamel of DF. And the enamel of DF to produce micropores was affected by acid etching and decalcification. DF is characterized by opaque, lusterless white patches in the enamel which may become striated, pitted and discolored with a breakdown of mineralized layer shortly after eruption.[9] With increasing severity of fluorosis, the subsurface enamel all along the tooth becomes increasingly porous (hypomineralized), and the lesion extends toward the inner enamel.[10] In our study, this finding was evident.

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 SEM and LCSM, so the relationship between the physical state of the enamel surface and the bond strength after acid etching was also very important. At the same time, from the viewpoint of mechanical bonding[8] , 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 there is extensive abrasion of the porous and soft exposed lesions in severe fluorotic enamel because when the well-mineralized surface zone is fractured away, the exposed hypomineralized lesions caused by extensive modifications in oral environment.[10] 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. [11]A recent study on ground sections of fluorosed teeth reported crescent-shaped hypomineralized areas in enamel and increased interglobular dentin spaces.[12]

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.[13, 14]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.[13, 15, 16] In addition, some scholars have shown that the bond strength of fluorosis tooth was lower than that of healthy tooth, the bond strength increased with the increasing concentration of phosphoric acid, but an excessively high acid concentration can conversely lead to an apparent decline in bond strength.[17] Therefore, in the case of enamel bonding of mild dental fluorosis, the bonding strength of the all acid etching adhesive was higher than that of the self-etching adhesive. The all acid etching bond strength of mild dental fluorosis was higher than the self-etched bond strength. However, there is no significant difference between the all etching bond system and the self-etching bond system of moderate and severe fluorosis. Because the fluoride content was significantly higher than normal teeth, it was observed under the SEM and LCSM that as the degree of dental fluorosis increased, the enamel structure became more disordered, and the effect of being formed microporous by acid etching and decalcification was worse, resulting in a decrease in bonding strength.

Conclusions

The bonding strength of mild fluorosis enamel to Prime & Bond NT was better than that of SE-Bond. And there is no difference in the bonding strength of SE-Bond to different degrees of DF.

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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