The long observation *in vitro* of prevention effect of novel self-etching orthodontic adhesive modified with 2-methacryloxyethyl phosphorylcholine in enamel demineralization

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The enamel demineralization is common in fixed orthodontics. Plaque accumulation around the bracket plays a critical role and could cause various degrees of white spot lesions (WSLs) on the surface of teeth. The 2-methacryloxyethyl phosphorylcholine (MPC) polymer is a biological polymer with protein repellent and an anti-bacterial adhesion effects. In this study, the enamel shear bond strength (SBS) and protein repellent property *in vitro* of self-etching orthodontic adhesive with MPC were evaluated. It was found that the self-etching adhesive with 0–7.5%MPC met the orthodontic clinical requirement on the SBS values at three different points of time. The incorporation of 7.5%MPC significantly reduced the bacterial adhesion and total microorganism of the yield biofilm. Moreover, the MTT assay showed that the amount of plaque metabolism in 7.5%MPC was the lowest among the groups. To conclude, the novel protein repellent self-etching adhesive was able to inhibit biofilm formation efficiently and minimize enamel demineralization.

Keywords: 2-methacryloxyethyl phosphorylcholine (MPC), Protein repellent, Self-etching adhesive, Enamel demineralization, Enamel bond strength

INTRODUCTION

Although orthodontic treatments would be able to correct malocclusion and improve facial aesthetics effectively, there are still adverse effects that occur in the process of orthodontic treatment. The self-cleaning function by soft tissue movement of the tongue and masticatory muscles could be hindered, leading to a high risk of enamel demineralization¹.

The process of enamel demineralization comprises of plaque accumulation, bacterial metabolic acid production, and acid erosion demineralization. Among them, accumulation of dental plaque is the most critical factor of enamel demineralization^{2,3}. Once the dental plaque has accumulated around the bracket, the cariogenic bacteria will metabolize carbohydrates to produce acid, which can lead to a decrease in pH value, and acid-resistant bacteria may increase in substantial quantities. After that, acid etching and demineralization on the enamel surface may arise. If white spot lesions (WSLs) could not be completely repaired by the self-mineralization of the system itself, for example, the natural remineralization of saliva to restore the enamel structure's normal morphology, caries could be finally formed, and the appearance of the teeth can be affected^{4,5}. Therefore, how to reduce the enamel demineralization has become a major issue for orthodontists.

Currently, there are three ways to prevent enamel

demineralization in orthodontic treatment: oral hygiene instruction, application of fluoride and standardization of all forms of orthodontic operations⁶. The effectiveness of self-maintaining oral hygiene, which is essential to avoid enamel demineralization, depends primarily upon the patient's cooperating. In regards of the application of fluoride, it has been shown to have the ability to resist enamel demineralization. However, fluoride has limitations, including being unsafe for children with dental fluorosis, reduced permeability and unreliable long-term effects, all of which have been considered in recent years^{7–9}.

It is well known that salivary proteins on the tooth surfaces can form an acquired pellicle, which is a prerequisite for bacterial attachment and plaque formation^{10,11}. Concerning orthodontic operation, the oversize enamel acid-etching and adhesive residual could lead to plaque and enhance the likelihood of enamel demineralization. Compared with the conventional 37% phosphoric acid etching, self-etching adhesive could reduce biofilm attachment and inhibit enamel demineralization. Our previous study proved that orthodontic adhesive with protein repellent property was able to avoid plaque attachment and WSLs, which is not reliant on patient compliance to prevent enamel demineralization.

Recently, the protein-repellent agent 2-methacryloxyethyl phosphorylcholine (MPC), a common biological complex with the function of protein repellent and anti-bacterial adhesion, has been utilized

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broadly in clinics. MPC polymer is one of the most common biocompatible and hydrophilic biomedical polymers. There is a lot of free water in water-containing MPC polymers, which could promote protein repellent effectively¹²⁻¹⁵.

In this study, we evaluated the effect of MPC on the protein repellent property of novel self-etching orthodontic adhesive.

MATERIALS AND METHODS

Incorporation of MPC into self-etching adhesive (AEO)

MPC powder (Sigma-Aldrich, St. Louis, MO, USA) was added into the self-etching adhesive, Adper Easy One (AEO, 3M, St. Paul, MN, USA), at the mass percentages of 0%, 3%, 5%, 7.5% and 10% respectively to prepare five groups of modified self-etching adhesive. The adhesive with MPC was magnetically stirred with a bar at a speed of 150 rpm for 24 h until the MPC in the AEO adhesive was completely dissolved.

A total of 180 premolars were collected from patients aged from 12 to 18, who had their premolars extracted due to orthodontic treatment with informed consent. The criteria of exclusion were teeth enamel hypoplasia, dental fluorosis and obvious invisible cracks. The 180 extracted teeth were randomly divided into six groups with 30 teeth per group. After the dental calculus on teeth and the soft tissue residuals were removed with a scaler, the teeth were then soaked in sterilized saline and immediately put into storage at 4°C. The tooth roots were embedded and fixed by a self-curing acrylic resin (New Century Dental Materials, Shanghai, China) in a 12×12×23 mm rectangular mold with the crown exposed for 10 mm.

The bonding method for bracket in each group was

as follows:

- (1) Acid-etch control, 37%phosphoric acid+resin modified glass ionomer cement (RMGIC);
- (2) Self-etch, AEO adhesive (referred to as AEO+0%MPC)+RMGIC;
- (3) Self-etch AEO adhesive+3%MPC (referred to as AEO+3%MPC)+RMGIC;
- (4) Self-etch AEO adhesive+5%MPC (referred to as AEO+5%MPC)+RMGIC;
- (5) Self-etch AEO adhesive+7.5%MPC (referred to as AEO +7.5%MPC)+RMGIC;
- (6) Self-etch AEO adhesive+10%MPC (referred to as AEO+10%MPC)+RMGIC.

Control group: The teeth were treated with a gel-like etchant containing 37% phosphoric acid (Scotch bond, 3M ESPE, St. Paul, MN, USA) for 30 s, and then rinsed thoroughly with a high-pressure water spray gun for 20 s. After that, the enamel surface was dried gently with an air gun, and then a light-curing RMGIC (Fuji Ortho LC, GC, Aichi, Japan) was used to bond metal premolar stainless steel brackets (OPA-K, Tomy, Fukushima, Japan).

Experimental Groups: The teeth belonging to group two to six were etched with AEO containing 0%, 3%, 5%, 7.5% and 10% MPC, respectively. Each modified paste was applied to buccal enamel surface to form a uniform coating by smearing for 20 s and drying for 5 s. RMGIC was used to fix the same brackets, as shown in Fig. 1.

Artificial saliva soaking and cold and hot circulation were commonly used to simulate complex oral environment for adhesive fatigue experiments. Two thirds of the teeth belonging to each group were immersed in 37°C artificial saliva for 30 or 180 days, respectively. After this, the specimens were taken out to go through 1,000 automatic thermal cycles

Fig. 1 Schematic diagram of orthodontic bracket specimens ($n=180$). Six different groups of tooth surface acid etching treatment, unified bonding of orthodontic stainless steel brackets, three different times (15 min, simulated oral environment 30 days, 180 days) to test the shear strength of brackets.

(TC501, Suzhou Weier, China) under the cold and hot temperatures (5°C and 55°C alternatively for 30 s) with a humidity difference^{16,17}.

Enamel shear bond strength (SBS) test and adhesive remnant index (ARI)

Within 15 min of bonding, the corresponding brackets were tested for SBS by Universal Material Testing Machine (AG-IS 500N, SHIMADZU, Kyoto, Japan). Each individual specimen was placed on an experimental machine and firmly screwed in place. In the decision making process, the cutting edge was placed on the bracket bottom plate, with the cutting direction being perpendicular to the top part of orthodontic bracket. The moving speed of the chisel was set at 0.5 mm/min. It was also ensured that the pressure chisel tip had a small friction force during the movement. When the bracket fell off, the computer would automatically records the load F (N) that the bracket borne at the moment of falling off. The shear strength P (MPa) or bonding strength was determined through the following formula: $P=F/S^{17}$, where S is the area (mm²) of each specimen adhered to bracket. The other groups were tested for SBS at two points of time (30 and 180 days after the cold and hot cycling).

The residual adhesive on the tooth surface was examined and scored *via* a stereoscopic microscope (Zoom-2330, WUMO, Shanghai, China) using the ARI^{17,18} at three points of time. The following scores were used: 0=no cement remaining on enamel; 1=less than half of the cement remaining on enamel; 2=more than half of cement remaining on enamel; 3=all the cement remaining on enamel, with a distinct impression of the bracket base.

Preparation of specimens

Based on the SBS results, 10%MPC groups had lower bonding strength that was not evaluated further. The other experimental groups (0%, 3%, 5%, 7.5%MPC groups) were evaluated further for the properties of protein adsorption and anti-bacterial tests.

The cover of 96-well plate (Costar, Corning, Corning,

NY, USA) was used for specimens preparation. For each specimen, the corresponding modified self-etching adhesive (experimental group) and AEO (control group) were applied to make a circular specimen with a diameter of 8 mm and a thickness of 0.5 mm as shown in Fig. 2. The specimens were immersed in the distilled water then stirred for 1 h to remove any uncured monomers¹⁹. After that, all specimens were dried and sterilized with ethylene oxide (Anprolene AN 74i, Andersen, Haw River, NC, USA), and store away from light²⁰.

Protein adhesion to the self-etch adhesive surface

Each group of specimens was placed into phosphate-buffered saline (PBS) for 2 h at room temperature, after which it was taken out and dried, then transferred onto the marked 24-well plates. Each well plate was injected with 2 mL of bovine serum albumin (BSA) solution (Sigma-Aldrich) at a concentration of 4.5 g/L with a pipette gun, and then soaked in the solution at 37°C for 2 h following a previous study¹⁹. The test specimens were then rinsed in fresh PBS, stirred at a speed of 300 rpm (Bellco Glass, Vineland, NJ, USA) for 5 min. And then those stored in 1% PBS with sodium lauryl sulfate (SDS), ultrasonically oscillated for 20 min. According to the micro bicinchoninic acid (BCA) protein assay kit²¹ (Pierce BCA protein assay, Thermo Scientific, Rockford, IL, USA), the standard reaction solution was taken based on the ratio of a:b=50:1 and injected onto 96-well plates with grouping marks, with 200 µL of reaction solution injected into each well. After that, 25 µL of BSA suspension from the vial holding the specimen after ultrasonic oscillation was taken out and added into each well. Under the condition of light protection, the 96-well plate was put into a 60°C oven for 30 min. The amount of protein adhered to the specimen was calculated for protein concentration¹⁹. Relevant data were recorded at a wavelength of 562 nm with a fully automatic quantitative mapping enzyme reader (SpectraMax Paradigm, Molecular Devices, Sunnyvale, CA, USA).

Saliva collection and dental plaque microcosm biofilm model

Human saliva has been shown to be an ideal source of *in vitro* plaque biofilm²². To culture human plaque biofilm for the inoculum, saliva was obtained from ten healthy adult donors who have natural dentition without active caries or periopathology, and who have also not used any form of antibiotics within the last three months²³. The donors collected their saliva 24 h from brushing their teeth and 2 h from drink and food intake before saliva donating²⁴. Saliva from each of the ten donors was combined to form the sample, and then diluted in sterile glycerol to a concentration of 70%, and then stored at -80°C²³.

Refer to the dental plaque culture method, the solution was mixed and placed in a measuring cylinder at the ratio of “human mixed saliva”: “Mcbain culture solution”: “20% sucrose”=2:100:1. After that, 1.5 mL of the sample was inoculated into the 24-well plate, which was then sealed and placed into a medical incubator

Fig. 2 Schematic diagram for preparation of test specimens.

at 37°C with 5%CO₂ content (Shanghai Fuma, China). After 8 h, 1 mL was sucked out from the upper part of the original culture medium and then was added to the 1 mL fresh inoculum (mixed solution with “McBain culture solution”: “20% sucrose”=100:1). After incubating for 16 h, replaced the inoculum the same way was done as mentioned above, and incubated all samples for another 24 h, then for a totaled two days^{25,26}.

Live/dead assay

All bacterial biofilm specimens cultured for two days were stained with live/dead kit (Molecular Probes, Eugene, OR, USA)^{24,26}. Afterward, the immersed specimens were dried and placed upside down into another well in 15 min, then analyzed for stained biofilm with fluorescence microscope (Eclipse TE2000-S, Nikon, Melville, NY, USA). Six specimens were evaluated for each group within 2 min. Three randomly-chosen view of fields were photographed for each specimen (10×10 magnification). The green living bacteria was then observed with a blue light at the 2nd gear, while the red dead bacteria was observed with a green light at the 3rd gear. The clear image was then removed.

MTT assay of metabolic activity

The aforementioned specimens covered by bacterial biofilm and cultured for two days were transferred to a new 24-well plate, with 1 mL of MTT dye (MTT dissolved in PBS solution, 0.5 mg/mL) added in each well and cultured at 37°C for 1 h in an incubator containing 5% CO₂ content. After this, the specimen was transferred to a new 24-well plate, with 1 mL of DMSO solution added in each well and gently stirred with no light at room temperature. The specimen was then cultured for 20 min and mixed evenly with a vortex mixing shaker (Silentshake, HYQ-3110, Shanghai, China). With all of this completed, 200 µL DMSO solution was drawn from each well and transferred to a 96-well plate covered with tin foil for an absorbance measurement of 540 nm by a microplate reader (SpectraMax Paradigm, Molecular Devices, Sunnyvale, CA, USA). The reading would also be recorded. A greater formazan concentration, which was related to a higher absorbance value, indicated a greater biofilm metabolic activity in the biofilm on the plate. MTT metabolic activity (A₅₄₀/cm²)=Biometabolic activity value (A₅₄₀)/Surface area of plate (cm²)^{24,27}.

Surface analysis by Fourier-transform infrared spectroscopy and X-ray photoelectron spectroscopy.

The functional group vibrations of the AEO sample without and with the MPC grafting were examined by Fourier-transform infrared (FT-IR) spectroscopy with attenuated total reflection (ATR) equipment. The FT-IR/ATR spectra were obtained using an FT-IR analyzer (FT/IR615, JASCO, Tokyo, Japan) for 32 scans (1.2 s/scan) over the range of 700–3,300 cm⁻¹ at a resolution of 4.0 cm⁻¹.

The surface elemental conditions of the AEO specimen without and with the MPC grafting were analyzed using X-ray photoelectron spectroscopy (XPS;

XPS, ESCALAB 250Xi, Thermo Fisher Scientific, Waltham, MA, USA) equipped with X-ray source with monochromatic Al target and double anode Al/Mg target.

Statistical analysis

One-way and two-way analyses of variance (ANOVA) were performed to detect the significance of the variables. Tukey's multiple comparison test was applied to compare the data at a *p* value of 0.05.

RESULTS

The results of enamel SBSs (mean±SD; *n*=10) were shown in Fig. 3. It can be observed that the bonding strength of the Group of acid-etch control was higher than those of other groups at the same time. The SBS of groups 2 to 5 had about 9.79–11.28 MPa. However, when the MPC concentration increased to 10%, the SBS decreased significantly (*p*<0.05). There was no statistical difference among the immediate shear bond strength in 15 min and the bracket shear strength after 30 days and 180 days in the first five groups (*p*>0.05).

Table 1 lists the ARI scores, indicating no significant differences among all the tested materials (*p*=0.99). The AEO containing up to 10% MPC did not alter the bonding process, as failure usually occurred at the bracket-

Fig. 3 Enamel shear bond strength results using extracted human molar (mean±SD; *n*=10).

The SBS decreased after incorporating 10%MPC into AEO compared to other groups (*p*<0.05). Meanwhile, the SBS had not been reduced significantly after immersion in artificial saliva for 30 days and 180 days plus thermal cycling compared with those at 15 min (*p*>0.05). The alphabet means the statistical analysis. The same letter (“a”) represents no significant intergroup difference, intragroup difference either. The different characters (“a”, “b” and “c”) represent significant intergroup differences (*p*<0.05). The symbol “bc” means that statistical significance either between 15min group and 30 days group, or between 30 days group and 180 days group was not found.

Table 1 ARI scores of orthodontic cements ($n=10$)

Group	Water-Aging	ARI Scores ^a			
		0	1	2	3
Acid-etch control	15 min	2	2	5	1
AEO+0%MPC	15 min	1	2	4	3
AEO+3%MPC	15 min	1	2	5	2
AEO+5%MPC	15 min	3	1	3	3
AEO+7.5%MPC	15 min	0	4	4	2
AEO+10%MPC	15 min	0	2	5	3
Acid-etch control	30 day	2	3	4	1
AEO+0%MPC	30 day	2	4	4	0
AEO+3%MPC	30 day	1	3	4	2
AEO+5%MPC	30 day	1	3	5	1
AEO+7.5%MPC	30 day	0	2	6	2
AEO+10%MPC	30 day	1	3	5	1
Acid-etch control	180 day	2	5	3	0
AEO+0%MPC	180 day	2	3	3	2
AEO+3%MPC	180 day	1	4	4	1
AEO+5%MPC	180 day	2	3	4	1
AEO+7.5%MPC	180 day	1	5	3	1
AEO+10%MPC	180 day	1	3	4	2

^a ARI was examined in accordance with the following scores: “0”=no cement remaining on enamel; “1”=less than half of the cement remaining on enamel; “2”=more than half of cement remaining on enamel; “3”=all the cement remaining on enamel, the failure site was between the cement and bracket base.

adhesive interface. There was no substantial variation between three times ($p>0.1$).

The BCA result of each plate demonstrated that the protein adsorption decreased with the concentration of MPC ($p<0.05$) (Fig. 4). The average protein adsorption value of group 3%MPC, 5%MPC and 7.5%MPC were $0.794 \mu\text{g}/\text{cm}^2$ (1 day) and $1.253 \mu\text{g}/\text{cm}^2$ (180 days), about 1/11–1/6 of that of the control group. It can be seen from Fig. 4 that the higher the concentration of MPC was, the stronger the protein repellent was, both at 1 day and 180 days after treatment. The long-term results of protein repellent in three modified groups had been slightly ineffective in terms of reducing protein adsorption compared with the 1 day results.

Representative live/dead images of 2 day biofilms grown on the disks are shown in Figs. 5: (A)–(D) represent images of biofilms on disks after being water aged one day. (E)–(H) represent images of biofilms on disks after being water aged for 180 days. As shown in Fig. 5, it is observable that AEO control was fully covered by a layer of primarily live bacteria (green). The amount of dental plaque in 0–5% MPC groups were much higher than that of live bacteria in the 7.5%MPC group from both results of 2 days and 180+2 days.

The area fraction of disk surfaces covered by live

Fig. 4 Histogram of protein adhesion onto self-etch adhesive disk surfaces in each group (mean±SD; $n=30$).

The adhesion in 3–7.5%MPC groups was significantly lower than that in the control group ($p<0.05$). The AEO with 7.5%MPC had far lower protein adsorption, which was about 1/18–1/12 of that of the control group. Bars with different letters indicate values that are significantly different from each other ($p<0.05$).

Fig. 5 Live/dead images of the biofilms on self-etch adhesive disks covered for two days. (A)–(D) represent images of biofilms on disks after being water aged 1 day. (E)–(H) represent images of biofilms on disks after being water aged for 180 days. Live bacteria were stained green, and compromised bacteria were stained red. When live and dead bacteria were in close proximity or on top of each other, the staining had yellow or orange colors. All group specimens were covered with major live bacteria. With the increase of concentration, the plaque distribution decreased as a result of the inhibition of salivary protein adsorption and bacterial adhesion.

Fig. 6 The area fraction of live bacteria on disks (mean±SD; $n=6$). There was no statistical difference between the two time points, marked by the same letter (“a”, “b”, “c” or “d”). Bars with dissimilar letters indicate values that are significantly different from each other. ($p<0.05$).

bacteria was plotted. Figure 6 showed that AEO+0%MPC control groups were almost 90% covered by a layer of mainly living bacteria. The higher the concentration of MPC, the lower the area fraction of live bacteria. There was no statistical difference within group ($p>0.05$). The sparsely distribution of plaque in the 7.5 MPC group indicated that MPC could perform a bacteriostatic effect.

Figure 7 showed the results of MTT assay on biofilm metabolic activity of 15 plates in each group. The biofilms on AEO+0%MPC control groups possessed the highest

Fig. 7 MTT metabolic activity of 2-day dental plaque microcosm biofilms on disks of self-etching adhesives (mean±SD; $n=15$). The biofilms on the AEO with 7.5% MPC showed the lowest metabolic activity among all the groups tested ($p<0.05$). Different small letters indicate values that are significant different from each other ($p<0.05$). There was no statistical difference between the two time points, marked by the same letter (“a”, “b” or “c”).

metabolic activity. The plaque metabolic quantity was decreased with the added amount of MPC. The average value of the 3%, 5%, 7.5% MPC group of specimens was 0.163 (2 days of culture), 0.167 (Saliva immersion 180 days+2 days of culture). 3%–7.5%MPC groups had also stability inhibited plaque metabolism following water aging ($p>0.05$).

Figure 8 showed the FT-IR/ATR spectra of the AEO sample and AEO+7.5%MPC sample. The peaks at 1,146, 885 and 980 cm^{-1} are attributed to the stretching vibration of P=O, P-O and C-O in the MPC graft polymer, respectively. The absorption region observed between 2,850 cm^{-1} and 2,950 cm^{-1} can be assigned to the N-H⁺ of the MPC. The bands in the 1,000–1,100 cm^{-1} region are attributed to the bending vibration of CH₃, CH₂ and the stretching vibration of C-N. It contains three vibrations at the similar region, so the absorption peaks are

Fig. 8 FT-IR/ATR spectra of the AEO sample (a) without and (b) with MPC grafting.

Fig. 9 XPS spectra (N_{1s} and P_{2p}) of the AEO sample (a) without and (b) with MPC grafting.

somewhat mixed and the peaks are not sharp. However, the band at 1,040 cm^{-1} , which was assigned to vibration of CH₂ in C=CH₂ group of the MPC structure, was not observed in the MPC-free AEO sample.

Figure 9 showed the XPS spectra (N_{1s} and P_{2p}) of AEO control specie and AEO+7.5%MPC experiment specie. In both N_{1s} and P_{2p} spectra, the peaks appeared only with MPC grafting specie. The peaks at 403 and 134 eV were assigned to the $-\text{N}^+(\text{CH}_3)_3$ and phosphate groups, respectively. These peaks were the characteristic of the phosphorylcholine present in the MPC units. The green curve belongs to the background counts when the XPS instrument is operating.

DISCUSSION

Acid-etch techniques are used routinely to bond orthodontic brackets to the teeth²⁸. In 1955, Buonocore initiated the acid etching technology and made a historical breakthrough²⁹. Fusayama *et al.*³⁰ first proposed the theory of total acid etching in 1979. In order to increase the brackets' bonding strength, the phosphoric acid solution was used for enamel surface treatment. 37% phosphoric acid etching has numerous drawbacks, including irreversibly removing several microns of the enamel layer, leading to enamel surface roughness, demineralization of the superficial layer of enamel near the brackets and junctions^{5,30,31}. In 1989, Chigira *et al.*³² proposed maleic acid and polyacrylic acid resins as substitutes for phosphoric acid in order to control excessive enamel loss.

The mechanism of the self-etching bonding theory to enamel is that the solution of acid etching primer can also reduce the excessive roughening of the treated enamel surface effectively, and simultaneously seal collagen fibers and hydroxyapatite crystals³²⁻³⁵, which is equivalent to forming a closed protective layer. Self-etching bonding theory could prevent the accumulation of bacteria plaque.

The key of plaque formation is the presence of acquired membranes on the surface of teeth or fillings, which are formed by the adhesion of salivary proteins. Studies have demonstrated that MPC could inhibit protein adhesion and present good biocompatibility¹³⁻¹⁵. It has also been shown that the filling materials (composite resins and dentin adhesives) with MPC can perform a strong anti-protein and anti-bacterial adhesion function. Therefore, self-etching adhesive could avoid enamel damage, simplify the operation and reduce the plaque formation, which could prevent the occurrence of enamel demineralization effectively. The premise of applying the self-etching adhesive with protein repellent function in clinic is that MPC modified self-etching adhesive requires stable bonding strength. This study investigated the effect of MPC on the bonding strength using self-etching adhesives with different MPC concentration, and laid the foundation for the development of self-etching adhesives with protein repellent properties.

Bonding strength of adhesives is essential in

orthodontic treatment. In general, orthodontic adhesives should provide sufficient bonding strength to resist the orthodontic and masticatory force. Multiple factors impact the bonding strength of brackets, such as the bracket floor area, the type of orthodontic adhesive, acid-etching technology, surface development and structural problems of the teeth. MacColl *et al.*³⁶⁾ reported that the shear bond strength testing area was independent from the bracket floor area, which was between 6.82 to 12.35 mm². In this study, a metal premolar stainless steel bracket (Tomy) with a base area of 9.94 mm² was selected to decrease the potential impact of the base area on the bonding strength. We also ruled out dental fluorosis and enamel hypoplasia in the exclusion criteria. Compared with traditional glass ionomer cement (GIC), RMGIC has higher bonding strength, wear resistance and smaller ARI index. Thus, RMGIC was used in our study. *In vitro* enamel bonding strength test is one of the predominant methods of evaluating the bonding strength. Reynolds³⁷⁾ suggested that the bracket bonding strength of 8–9 MPa meet clinical requirements. In our study, the bonding strength of all the groups in this study was no more than 13 MPa. ARI scores indicated no significant variations among all the conditions tested, and failure usually occurred at the bracket-adhesive interface.

The oral environment is relatively complex. The oral adhesive area is under the humid environment, and the internal temperature of the oral cavity fluctuates greatly with the dietary temperature. The temporary bracket bonding in orthodontic treatment is estimated 1–3 years, which requires sufficient bonding force to prevent bracket bonding failure. Meanwhile, the easy removal of adhesive from the tooth surface after orthodontic treatment is also essential. After orthodontic treatment, a 10-fold magnifying glass is typically utilized to observe the cracking situation on the enamel surface, because the bracket bonding strength is sometimes extreme high when the bracket is removed. The average bonding strength is 15.288 MPa, which results in excessive force on the tooth surface. Lamper *et al.*³⁴⁾ and Retidf³⁸⁾ found that little enamel damage was noticed when the bond strength was lower than 12 MPa, however, the risk would increase 14-fold when the strength was over 12 MPa. When the bonding strength reached 13.5 MPa, the glaze would be destroyed. Therefore, low bonding strength could affect orthodontic treatment, but high bonding strength could damage enamel surface during removal of appliance. Our SBS results showed for the group two to five that the values were higher than 9.79 MPa and lower than 11.28 MPa. Hence, the modified self-etching adhesive could meet the requirements on bracket bonding strength and avoid tearing or cracking enamel surface when the brackets are removed. In our long-term study, the coefficient of thermal expansion of enamel, AEO, RMGIC and bracket could be different, resulting in uncoordinated alterations in internal stress. There could be a micro-leakage between the materials, so the bond strength of 180-day aging might be slightly lower or similar to those of shorter two times. However, 180-day bonding strength could also be acceptable in a

clinic environment.

Regarding the long-term durability of the protein repellent and antibacterial properties, the attachment of oral bacteria to a material surface is mediated by adsorbed proteins, and protein coating is the first necessary step for bacteria attachment^{11,39)}. Amphoteric MPC is a methyl acrylate with side chain of phospholipids as the polar group¹⁵⁾. Phospholipid is a kind of lipid, and can form a phospholipid bilayer. The structure of phospholipid is generally composed of a hydrophilic head (attracted by water) and a hydrophobic tail (repelled by water). There is a substantial quantity of free water in the water-containing MPC polymers, but no binding water. Free water can effectively resist protein adhesion, and inhibit the adhesion of salivary protein and plaque formation. MPC was reported to contain reactive methacrylate groups which could be co-polymerized and covalently-bonded with the resin matrix upon photo polymerization⁴⁰⁾. A previous study demonstrated that MPC was co-polymerized with acrylic resin through covalent bonding, and the strong C–C bonding offered durable resistance to protein adsorption⁴⁰⁾. Zhang *et al.* reported that the SBMP primer and adhesive contained HEMA (hydroxyethyl methacrylate and phosphorylated methacrylate) and a copolymer of acrylic/itaconic acids, which could co-polymerize with MPC⁴¹⁾. We used infrared spectroscopy to explore the specific chemical bonding mode of the modified materials, and the XPS method was adopted to determine whether the MPC polymer was on the modified adhesive. Our study found that both immediately and 180 days after simulating the oral environment, the specimens could confirm the existence of MPC. These results showed that the specimens with MPC could know the obvious combination of different peaks and chemical bonds in “P” and “N” regions. In this study, the self-etching adhesive component contains HEMA⁴²⁾, and it could be photo-cure copolymerized with the methacrylate group in the MPC monomer, and MPC could be fixed into the self-etching adhesive realizing an everlasting anti-protein effect. Based on above considerations, in the present study, protein repelling property of the modified self-etching adhesive were tested using the micro bicinchoninic acid method. The protein adsorption in 7.5%MPC group was 0.486±0.14 µg/cm² (1 day) and 0.686±0.19 µg/cm² (180 days), about 1/18–1/12 that of the control group.

A human saliva microcosm biofilm model was used to investigate the biofilm formation and its metabolic activity by live/dead assay and MTT assay, which could offer a new idea for the enamel demineralization prevention during orthodontic treatment. When culturing dental plaque biofilm, the environment maintained stable, and the artificial saliva bacteria were strictly standardized according to the requirements every time. After culturing plaque biofilm for two days, we found that the turbidity of the culture medium varied. The greater the concentration of MPC was, the less the turbidity would be. In the 7.5%MPC group, the orifice plate was clear and visible at the bottom. The quantity of bacteria on the surface of specimens decreased with

the increase of the MPC concentrations. The qualitative and quantitative analyses of anti-microbial activity were consistent. Our study found that the plaque biofilm in 7.5%MPC group showed significant decrease. Therefore, 7.5%MPC had the strongest inhibition effect on plaque metabolism, hence the novel protein repellent self-etching adhesive was promising to inhibit biofilm formation.

Our study still requires further *in vitro* observation (for longer than 180 days), with expanding sample size. Our results revealed that the higher the MPC concentration, the higher anti-protein function. While, the higher MPC concentration, such as 10% MPC had lower enamel shear bond strength which could not be used in clinic. With all results taken into consideration, we suggested that 7.5% MPC contents could both satisfy clinically acceptable enamel bond strength and plaque repelling function. To find the best MPC concentration, more MPC concentrations should be tested in further study. Besides, the morphological observation of tooth surface after acid etching of modified materials should be investigated before clinical application. It is also critical to further examine the chemical structure and chemical states of the functional groups on the surface of the materials before and after the addition of MPC to AEO.

CONCLUSIONS

The self-etching adhesive with MPC could perform sufficient protein repellent function, reduce plaque formation and present anti-bacterial capabilities. Within the limit of this study, the 7.5%MPC appears to be an optimal proportion in obtaining the strongest protein repellent function without compromising the SBS. The modified self-etching adhesive can provide a new idea for enamel demineralization prevention and related clinical operations optimization in orthodontic treatment.

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