Effect of Exposure Times of Sodium Hypochlorite before Acid Etching on the Microshear Bond Strength to Fluorotic Enamel

Zhengfan S * / Meifeng Z ** / Wei P *** / Bo Z **** /Ling G *****

Purpose: To evaluate the effects of different treatment time of 5.25% Sodium hypochlorite (NaOCl) on the microshear bond strength (μSBS), attenuated total reflection Fourier transform infrared (ATR-FTIR) and etching pattern in mild and moderate fluorotic enamel. Study design: Forty-eight fluorotic molars were divided into two groups: mild and moderate fluorotic enamel which were classified by a Thylstrup and Fejerskov index (TFI). Based on the application time (0s, 60s, 120s, 180s) of 5.25%NaOCl, each group was sectioned into four parts. Then the etched enamel was bonded with resin and tested to acquire μSBS. The statistical method was two-way ANOVA and Least Significant Difference (LSD) test at α = 0.05. Besides, fracture modes were observed under a stereo microscope. SEM was used to evaluated the enamel-etching pattern and organic content on the fluorotic enamel surface were investigated by ATR-FTIR. Results: Duration of 5.25%NaOCl at 60s or 120s significantly increased the μSBS of fluorotic enamel compared to 0s ($p<0.05$). Fracture modes indicated that dominating failures were set in the bonding interface but whose proportion decreased when 5.25%NaOCl was applied. The enamel-etching pattern in 180s was deepest under SEM. Spectra of enamel samples manifested an obvious and gradual removal of its organic phase after duration of NaOCl increased. Conclusion: The maximal μSBS is acquired by using 5.25%NaOCl at 60s for mild fluorotic enamel but 120s for the moderate. The prolonged application time of 5.25%NaOCl prior to phosphoric acid etching improves enamel-etching pattern. Treatment of 5.25%NaOCl decreases proteins on the fluorotic enamel surface.

Keywords: Fluorotic enamel, Microshear bond strength, Sodium hypochlorite, Etching pattern

INTRODUCTION

Because of successive exposures to high concentrations of fluoride during tooth development, dental fluorotic enamel is disturbed, which shows hypomineralized and porous.[1] Clinically, bilateral, diffuse (not sharply demarcated), opaque, and white lines following the perikymata were seen on the appearance of mild fluorotic enamel. The white patches may be formed by opacities. Severe fluorotic enamel may become entirely chalky-white, discolored from light to dark brown and/or pitted.[1, 2] According to biological aspects of dental fluorosis, TFI is used to be classified on a scale of one to nine: mild (TFI = 1-3), moderate (TFI = 4-5) and severe (TFI = 6-9). [1,3, 4]

Although dental fluorosis might not affect our oral health seriously, it compromises the tooth's aesthetics and brings potential psychosocial effects on many patients who experience social repercussions.[5, 6] Therefore, dentists have been proposed a broad range of therapies of various invasiveness to treat fluorotic enamel, including resin infiltration,[7] microabrasion,[8] dental veneers,[1] external bleaching,[9] crowns[10] or a combination of these methods.[11, 12] Because fluorotic enamel has high resistance in acid etching and the hypomineralization of enamel region has the porous nature, the shear adhesive strength of fluorotic enamel is lower than that of normal enamel.[6] Hence, how to increase the bonding strength of fluorotic enamel must be considered in most restorative procedures.

From the School of Stomatology Southwest Medical University, Department of Prosthodontics, Hospital of Stomatology Southwest Medical University, Luzhou, China.
* Zhengfan Sun, Graduate student.
** Meifeng Zhang, Graduate student.
*** Wei Peng, Graduate student-
**** Bo Zhang.
*****Ling Guo, Professor.

Corresponding author:
Ling Guo, Professor, Department of Prosthodontics, Hospital of Stomatology Southwest Medical University, No. 2 Jiangyang West Road, Luzhou 646000, Sichuan, China
E-mail: 372083745@qq.com

Some studies suggested that application with 5.25% NaOCl could increase enamel surface area before acid etching which is suitable for resin bonding.[13, 14] NaOCl has been known that it is efficient to remove organic matter at common temperature as a nonspecific proteolytic agent.[13, 15] 5.25% NaOCl solution has been applied to remove organic detritus which come from pulp canals in clinic. Additionally, some recent studies reported that NaOCl was applied to dissolve collagen before dentin bonding.[16, 17] The reason that NaOCl improves bonding strength is that it removes protein from surface of enamel.[15, 18]

There are conclusions that good bonding results have been obtained on hypomineralized and normal enamel which applied 5.25% NaOCl.[15, 18] Besides, a recent study has found that treatment with 5.2%NaOCl before fluorotic enamel(TIF=4) acid etching increased orthodontic brackets' bond strength.[19] However, most research showed that the duration of 5.25% NaOCl was 60s without giving a reason. To date, only Roberto et al evaluated that deproteinization with 5.25% NaOCl could increase μSBS before etching but processing time at 60s was more effective than 30s in enamel.[14] Whereas, there was a remarkably greater protein content by weight in fluorotic enamel compared with the normal.[20] We have to assume that extending pretreatment time of 5.25% NaOCl whether have an beneficial effect on bonding strength or not and whether there are differences in the processing time of 5.25% NaOCl between mild and moderate fluorotic enamel.

In consequence, this experiment was aimed at comparison between the μSBS of mild and moderate fluorotic enamel after pretreatment with 5.25%NaOCl at different times and make further exploration of enamel-etching pattern and ATR-FTIR. The null hypotheses tested that various exposure times with 5.25% NaOCl will make no difference to μSBS, etching pattern of enamel or ATR-FTIR of mild and moderate fluorotic enamel.

MATERIALS AND METHOD

The Human Ethics Committee that come from the School and Hospital of Stomatology, Southwest Medical University approved this study.

Forty-eight fluorotic molars were extracted from patients due to severe periodontal diseases after getting an informed consent which was taken to use the teeth in the research. According to teeth's unique clinical feature, dimensions, morphology, appearance without any cracks or carious cavities, the fluorotic teeth were selected. Two examiners were trained and dental fluorosis was divided into two categories according to the TFI: mild dental fluorosis (ML-F) and moderate dental fluorosis (MD-F). Then, the periodontal film and dental calculus were removed and cleaned before teeth were stored at 4°C and in 1% thymol solution. Besides, teeth must be used within 6 months.

Gypsum was used to immobilize roots of teeth in mould and each dental crown was then cut it into four pieces which were in parallel with the long axis of teeth (buccal surface, lingual surface and two proximal surfaces), for 192 enamel surfaces in total: 168 enamel surfaces were used to evaluate μSBS, 16 were used for the etching pattern and 8 were used for ATR-FTIR analysis.

The samples in ML-F and MD-F group were respectively separated into 4 subgroups (n=21 per subgroup) based on application times (0s, 60s, 120s and 180s) of 5.25%NaOCl. Specimens were embedded in Polymethyl methacrylate through silicone mould (10 mm long × 10mm wide × 5mm high) (LELE, Shanghai, China). In order to acquire a smooth flat of enamel, using 600 grit wet silicon carbide sandpaper polished exposed enamel surfaces for 30 seconds. Each subgroup was treated as follows:

0s-group washed with water and then dried with oil-free compressed air for 10s.[21] (control group)

60s-group treated with 5.25% NaOCl by applying sterile cotton pellet for 60s, washed with water and then dried with oil-free compressed air for 10s.

120s-group treated with 5.25% NaOCl by applying sterile cotton pellet for 120s, washed with water and then dried with oil-free compressed air for 10s.[13, 14]

180s-group treated with 5.25% NaOCl by applying sterile cotton pellet for 180s, washed with water and then dried with oil-free compressed air for 10s.

Microshear Bond Strength Test

A single operator performed detailed application methods of 5.25%NaOCl as shown above. Subsequently, 35% phosphoric acid gel (*Gluma, Hanau, Germany*) etched enamel surfaces for 30s. Then, water washed it and oil-free compressed air dried it for 10s.[13] The polished enamel surface was put a piece of acid-resistant, double-faced adhesive tape with two to three perforations that were 1.1mm in diameter. The number of perforations depended on the area of enamel surface. The adhesive (*Single Bond Universal, 3M, Saint Paul, USA*) was applied to the enamel surface according to the instructions. Then transparent tygon tubes (*Oudelixin, Shanghai CHINA*) whose diameters were the same as perforations and whose heights were 1 mm were put on the double-faced tape, guaranteeing that their lumen was aligned with the circular areas of perforations.[22] Composite resin (Filtek Z350, 3M, Saint Paul, USA) was meticulously filled in each tube. Using a LED light-curing lamp (Woodpecker, Guangxi, China) cured resin composite for 20s.

Specimens were stored in deionized water for 24h at 37°C. Whereafter, a blade removed the tygon tubes with caution and the double-faced adhesive tape carefully in order to expose the composite cylinders. The stereomicroscope examined specimens under at 10× magnification. If bonding interface has porosities or gaps, the bonded cylinder was discarded.[2] Specimens were fastened with a universal testing machine (*WDW20, YINUO, Jinan, China*) and the base of each composite cylinder was looped by a thin orthodontic wire (0.2 mm diameter). The composite resin cylinder contacted with the orthodontic wire in half of its circumference and specimens were stressed at 1 mm/min until fracture. The μSBS values (MPa) were recorded by machine.[22] Subsequently, a stereo microscope *(Motic, Carlsbad, CA*) which was at 40× magnification was used to observed fracture mode, which was categorized into adhesive (Mode A), cohesive in enamel or in resin (Mode B), mixed (Mode C).[23]

Enamel etching pattern

The enamel-etching pattern (n = 2 enamel surfaces per subgroup) was tested by using a electron microscope. After applying the treatment of 5.25% NaOCl at different times, then 35%H_3PO^4 gel etched the enamel surface for 30s. Finally, water washed it and dry air sprayed it for 10s. All enamels were dehydrated for 12h, coated with a thin layer of gold by spraying and observed by an Inspect F50 (*FEI Co., thermo fisher*) that magnified the object 5000 times.

Attenuated total reflection Fourier transform infrared

4 mild and 4 moderate fluorotic slabs were used to ATR-FTIR analysis. Slabs were embedded in an acrylic resin and then were trimmed to about 8 mm × 8 mm × 2 mm.[24] Water-wet silicon carbide paper (up to 1 000 grit) polished each embedded enamel. Then, enamel was ultrasonicated in distilled water for 5 min to remove residual debris.[17] The specimens were then put on the surface of the Ge crystal which was the smart OMNI sampler accessory. Each enamel slab was randomly measured two selected and marked locations.[17, 24, 25] Spectra were collected in the range from 700 to 4,000/cm^{-1} at 4/cm^{-1} resolution by using 100 scans.[26] This process was a self-controlled study before and after 5.25%NaOCl treatment for 60s and sequentially repeated the previous step so that spectra were recorded at total time of 0s, 60s, 120s and 180s.[16] Besides, the spectrum of air were automatically subtracted by the OMNIC 7 software. The results of NaOCl on deproteination at various time was finally compared after baseline correction and normalization,.[17, 27, 28]

Statistical Analysis

For each sample, dates from μSBS were evaluated by two-way ANOVA and the LSD test was used to analyze statistic differences between 2 subgroups. Using SPSS (*SPSS Inc, Chicago, IL*) to analyze the data whose level of statistical significance was set at 0.05. Fracture mode data was showed with GraphPad Prism 5.0 software (*GraphPad Software, La Jolla, CA*).[23] The spectra was dealt with Origin 2021 software (*OriginLab, Massachusetts, USA*).

RESULTS

Microshear Bond Strength

As shown in table 1, the μSBS of each group is presented. The different severity of mild and moderate dental fluorosis had not significant effect on the μSBS when treated with 5.25%NaOCl at same time. However, the various duration of NaOCl had obviously different μSBS in mild or moderate fluorotic enamel. A significant difference was observed between 0s-group and 60s-group ($p < 0.01$). At the same time, 120s-group significantly increased the bond strengths compared to 0s-group as well ($p < 0.01$). Conversely, the μSBS at 180s was the lowest in all groups and showed a statistically significant decrease compared to 0s–group in ML-F group ($p < 0.05$). When 60s-group and 120s-group were compared, statistically significantly higher mean bond strengths were obtained to 120s-group than to 60s-group ($p < 0.05$) in MD-F group. But in ML-F group, there was no difference between 60s-group and 120s-group ($p > 0.05$). Table 2 showed the fracture modes of each group and the majority of fracture modes was in Mode A. Nevertheless, Figure 1 showed the occurrence rate of facture in Mode A decreased as 5.25%NaOCl was applied.

Table 1: Microshear bond strengths in MPa (means ± standard deviations) of the different groups

Group	0s	60s	120s	180s
ML-F	25.55± 4.8^{a}	28.40 ± 4.0^{b}	29.31± 4.2^{b}	22.98 ± 4.0^{c}
MD-F	25.12 ± 3.6^{A}	28.02 ± 3.8^{B}	30.63 ± 3.9^{C}	24.63± 3.9^{A}

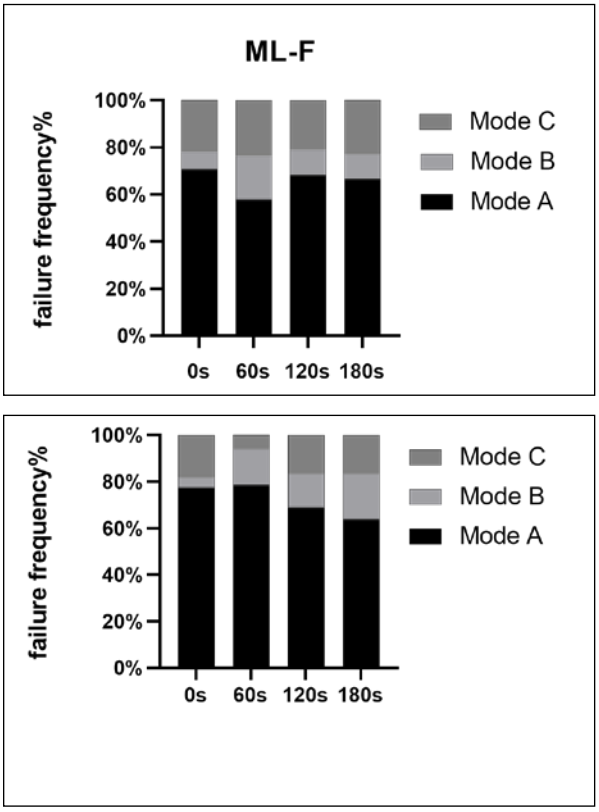
Note: For each line, different superscript letters indicate statistically significant differences between groups (two-way ANOVA; LSD test, $p < 0.05$). For each column, there is no statistical difference ($p > 0.05$).

Table 2: Number of specimens according to fracture mode* for all experimental groups

Group (facture mode)	ML-F			MD-F		
Time	A	B	C	A	B	C
0s	25	2	6	25	1	7
60s	21	5	7	25	3	5
120s	24	2	7	24	4	5
180s	23	2	8	19	6	8

* A–adhesive; B–cohesive in enamel or resin; C–mixed;

Figure 1: Fracture modes (%) of ML-F and MD-F groups (Mode A: adhesive, Mode B: cohesive in enamel or resin, Mode C: mixed)



SEM observation

The representative SEM image of the enamel-etching pattern in each group are shown in Figure 2. Moderate dental fluorosis who compared the mild, after deproteinization with NaOCl and acid-etching, showed more dissolution of prisms cores in the same processing time. While more prism peripheries dissolve with the increase of NaOCl application time at the same degree of fluorotic enamel. An increase of irregularities and micro-porosities on the fluorotic enamel surface was visible compared to enamel with the 0s-groups. 180s-group revealed a more remarkable etching pattern with more underlying enamel compared with 60s or 120s. So deproteinization and acid-etching increased the porosity of enamel surface whose etching patterns showed more profound.

Attenuated total reflection Fourier transform infrared (ATR-FTIR)

FTIR spectra showed major chemical groups in both mild and moderate fluorotic enamel (Figure 3): the phosphate ν_1, ν_3 stretching mode (900–1200 cm^{-1}), carbonate ν_3 stretching mode (1350–1520 cm^{-1}) and carbonate ν_2 deformational mode (845–890 cm^{-1}) from the mineral component, the amide I bands (1544 cm^{-1}) and amide II bands (1637 cm^{-1}) from the organic component.[28, 29] The spectra showed that mild and moderate fluorotic enamel were treated with different duration of NaOCl (Figure 4). Apatite is undissolving in NaOCl so the intensity of phosphate stretching vibration peak is constant.[16, 17] Therefore, we normalized the spectra to the phosphate ν_3 (peak at 985/cm^{-1}) in Figure 3 and Figure 4.

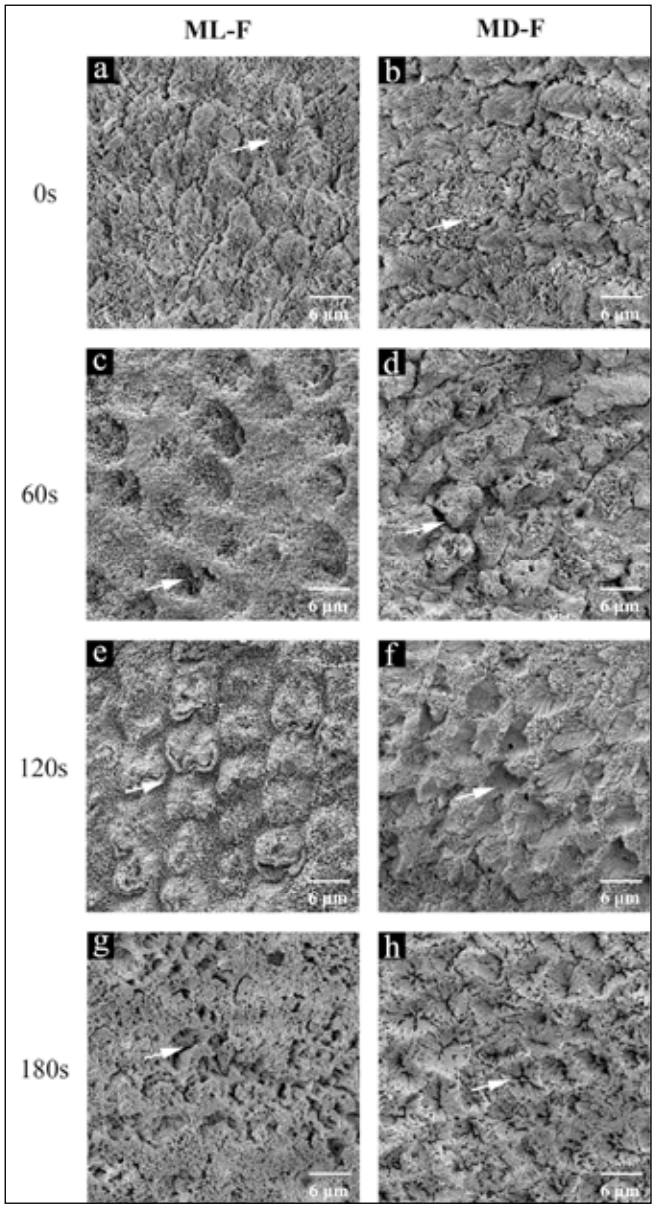
As can be seen, the amide I and amide II bands are significantly larger in spectra of our moderate fluorotic enamel samples than the mild and so does carbonate ν_3 band (Figure 3). These spectra showed an obvious weakening of the peaks at 1,550, and 1,643/cm^{-1} after NaOCl treatment (Figure 4). The presence of phosphate peak became more legible with prolonged time (Figure 4). With increased exposure time of NaOCl, the band at 1,643/cm^{-1} showed weaker. But in 60s, 120s and 180s, the amide bands seemed to overlap in the ML-F group and the peaks at 1,643/cm^{-1} in the MD-F group was relatively distinct. The differential spectra clearly indicate a decrease in the organic content with the application of NaOCl or an increase in the organic content with the severity of dental fluorosis.

Figure 2: SEM images of various groups at a magnification of ×5,000. (ML-F: mild fluorotic enamel, MD-F: moderate fluorotic enamel). Mild and moderate fluorotic enamel treated with 5.25%NaOCl for different treatment times (0s, 60s, 120s, 180s).

Fig. a, b: Enamel rods and inter-rod substance with a nearly uniform etching pattern but areas of hypomineralization of enamel can also be observed (white arrow).

Fig. c, d, e and f: the inter-rod substances were removed and both type I etching (in which the enamel rod, or prism, head are dissolved) and type 2 etching (in which the enamel interprismatic substance is dissolved) patterns are evident (white arrow).

Fig. g, h: barely visible enamel rods and irregular etching pattern were shown (white arrow).



DISCUSSION

The results of the present study indicated that the deproteinization of 5.25% NaOCl at 60s or 120s significantly increased bond strength than that at 0s in mild and moderate fluorosis. The optimum treatment time of 5.25% NaOCl was at 60s in ML-F group but 120s in MD-F group. There were obvious changes in bonding strength of fluorotic enamel after various duration of pre-treatment with NaOCl solution, so the null hypothesis that "various exposure times with 5.25% NaOCl will not influence the μSBS." can be rejected. Besides, the longer the treatment time of NaOCl in 0 s to 180s was, the more profound etching patterns were under SEM. The spectra of enamel demonstrated that organic content decreased after NaOCl was applied. Hence, the hypothesis that 'various exposure times with 5.25% NaOCl will not influence enamel-etching pattern and the ATR-FTIR of fluorotic enamel.' was also rejected.

Besides, it is consistent with results from Mariana et al, in which the treatment of 5.2%NaOCl at 60s markedly increased bonding strength to fluorotic enamel.[19] we qualitatively analyzed

Figure 3: A typical ATR spectra of mild fluorotic enamel (ML-F) and moderate fluorotic enamel (MD-F) without NaOCl treatment. The chemical components on enamel whose absorption peaks were between 700 and 2,000 cm^{-1} are shown.

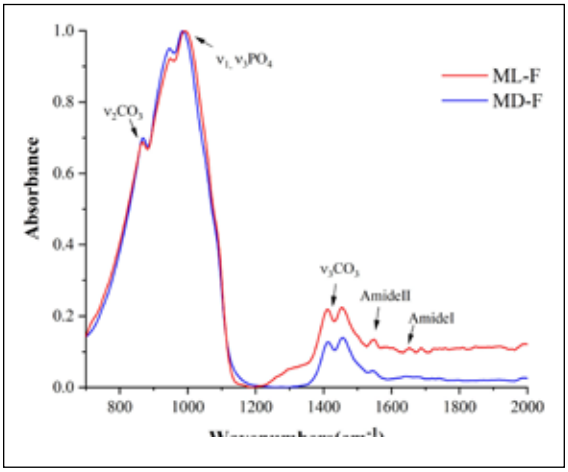
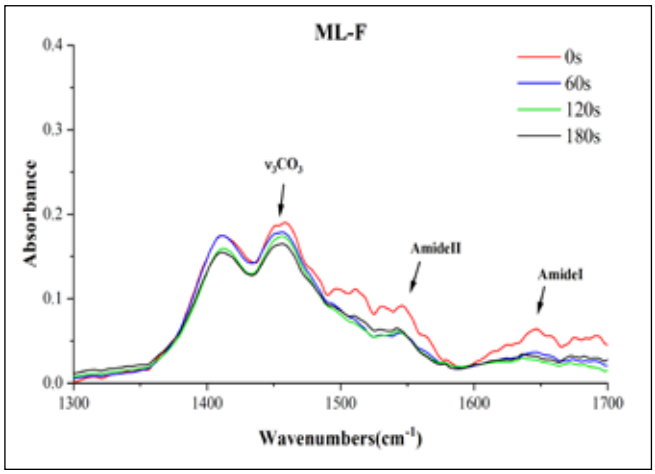
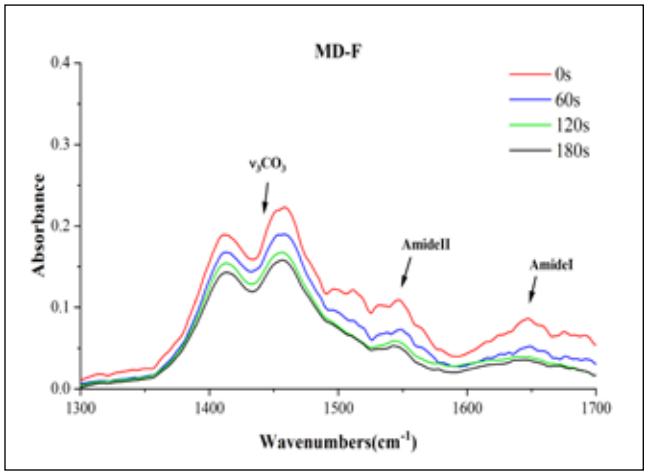


Figure 4: Representative ATR spectra of mild fluorotic enamel (ML-F) and moderate fluorotic enamel (MD-F) after 5.25%NaOCl treatments with different exposure times. The red, blue, green and black lines represent ATR spectra of enamel after NaOCl treatments for 0s, 60s, 120s, 180s groups, respectively.





the spectra on the amide bands and carbonate bands by ATR-FTIR and discovered that 5.25% NaOCl did indeed decrease protein content in enamel surface. This result is consistent with the findings of others studies.[16, 17]

At the same time, what we found under SEM was consistent with Valencia et al that the center of the prism crystals was more easily attacked without NaOCl deproteinization, while the inter-rod substance was affected with NaOCl deproteinization.[30] Because inter-rod areas contain more organic material than enamel rods.[30] It was known that application with 5.25% NaOCl could be used to increase the bonding area between composite material and the tooth surface before acid etching. [13, 21, 30, 31] The type 1-2 etching pattern was increased when deproteinization with 5.25% NaOCl was used for enamel at 60s.[14, 21, 30] This could be used to explain application with 5.25% NaOCl can increase bond strengths for dental fluorosis.

In ML-F group, there is no difference in bond strength between 60s-group and 120s-group. However, in MD-F group, bond strengths of 120s-group were significantly increased than that of 60s-group. The results of spectrum showed that moderate fluorotic enamel surface had larger protein content than that mild (Figure 3). In addition, the amide bands which was in 60s and 120s seemed to overlap in the ML-F group but in the MD-F group, the band in 120s was distinctly weaker than that in 60s. Therefore, we speculated that MD-F needed a longer processing time of 5.25% NaOCl so that can remove more enamel surface protein. However, area integration of bands originating from carbonate and proteins was difficult to quantitative analysis due to broad or overlapping bands because processing time was too short.

However, the result indicated that treat time with 5.25% NaOCl before adhesive application was not the longer the better. Conversely, it has a specified time range. Time likes 60s or 120s would be beneficial but not 180s.The study found that the μSBS at 180s was the lowest in all groups and even showed a statistically significant decrease compared to 0s–group in ML-F group ($p < 0.05$). What's more, SEM revealed that the 60s-group and 120s-group whose enamel rods and inter-rod substance were unambiguous and more uniform than 180s-group. On the contrary, 180s-group showed obscure enamel rods and anomalous etching pattern which was in a more pronounced etching pattern with more exposure of the porosity of enamel surface compared with 60s-group and 120s-group under SEM (Figure 3). The previous findings indicated that prolonged application of 5.25% NaOCl might increase porosity of enamel.[19] Thus we assumed that increased porosity which indicated less and less normal enamel rods structure led to bonding strength decreased.

In addition, many literatures suggest that NaOCl produces reactive free radicals which inhibit resin polymerization adequately.[19, 32] Because these residual free radicals compete with the propagating vinyl free radicals generated during light activation, it leads to premature chain termination and partial polymerization. [2,19] Therefore, we speculate that the overlong application time of 5.25% NaOCl could have more free radicals on the increased enamel porosities which can't be removed by rinsing completely and decreasing the conversion degree of the enamel-resin cement interface may result in a lowering of bond strength. Hence, we can go on with the experiment to explore in situ degree of conversion of adhesive after different time of 25% NaOCl application.

In summary, 5.25%NaOCl as an alternative deproteinization agent is beneficial for bonding to fluorotic enamel on the basis of the results. But the low surface energy of fluorotic enamel which has been verified more porous and hypomineralized impairs surface wetting and the surface with well outer mineralization is quite fragile in severe cases.[19, 23, 33] Further studies are needed to test the bonding strength involving thermocycling, resin penetration, in situ conversion degree of the resin cement and the mechanism of enamel surfaces after deproteinization by NaOCl.

CONCLUSION

The present study drew from these following conclusions. The maximal μSBS is acquired by using 5.25%NaOCl at 60s for mild fluorotic enamel but 120s for the moderate. The prolonged application time of 5.25%NaOCl enhanced enamel-etching pattern and minimize superficial enamel protein level. 5.25%NaOCl could be an alternative deproteinization agent for bonding dental adhesive to fluorotic enamel.

REFERENCES

1. Abanto Alvarez J, Rezende KM, Marocho SM, Alves FB, Celiberti P, Ciamponi AL. Dental fluorosis: exposure, prevention and management. Medicina oral, patologia oral y cirugia bucal. 2009; 14(2):E103-7.
2. Siqueira FSF, Armas-Vega A, Izquierdo-Bucheli A, et al. Does the Conditioning Mode and Duration of Universal Adhesives Affect the Bonding Effectiveness to Fluorotic Enamel? J adhesive dent. 2019:21(6):525-536.
3. Clarkson J. Review of terminology, classifications, and indices of developmental defects of enamel. Advances in dental research. 1989; 3(2):104-9.
4. Thylstrup A, Fejerskov O. Clinical appearance of dental fluorosis in permanent teeth in relation to histologic changes. Community dentistry and oral epidemiology. 1978; 6(6):315-28.
5. Di Giovanni T, Eliades T, Papageorgiou SN. Interventions for dental fluorosis: A systematic review. J esthetic restorative dent : official publication of the American Academy of Esthetic Dentistry. 2018:30(6):502-508.
6. Gu M, Lv L, He X, Li W, Guo L. Effect of phosphoric acid concentration used for etching on the microtensile bond strength to fluorotic teeth. Medicine. 2018; 97(35):e12093.
7. Cocco AR, Lund RG, Torre E, Martos J. Treatment of Fluorosis Spots Using a Resin Infiltration Technique: 14-month Follow-up. Operative dentistry. 2016; 41(4):357-62.
8. Khandelwal V, Nayak UA, Nayak PA, Ninawe N. Aesthetic management of dental fluorosis. BMJ case reports. 2013.
9. Loyola-Rodriguez JP, Pozos-Guillen Ade J, Hernandez-Hernandez F, Berumen-Maldonado R, Patiño-Marin N. Effectiveness of treatment with carbamide peroxide and hydrogen peroxide in subjects affected by dental fluorosis: a clinical trial. 2003; J Clin Ped Dent. 28(1):63-7.
10. Akpata ES. Occurrence and management of dental fluorosis. Intern dent J. 2001; 51(5):325-33.
11. Castro KS, Ferreira AC, Duarte RM, Sampaio FC, Meireles SS. Acceptability, efficacy and safety of two treatment protocols for dental fluorosis: a randomized clinical trial. J dent. 2014;42(8):938-44.
12. Gugnani N, Pandit IK, Gupta M, Gugnani S, Soni S, Goyal V. Comparative evaluation of esthetic changes in nonpitted fluorosis stains when treated with resin infiltration, in-office bleaching, and combination therapies. Journal of esthetic and restorative dentistry : official publication of the American Academy of Esthetic Dentistry [et al]. 2017; 29(5):317-324.
13. Christopher A, Krishnakumar R, Reddy NV, Rohini G. Effect of Enamel Deproteinization in Primary Teeth. J Clin Ped Dent. 2018; 42(1):45-49.
14. Espinosa R, Valencia R, Uribe M, Ceja I, Saadia M. Enamel deproteinization and its effect on acid etching: an in vitro study. J Clin Ped Dent. 2008; 33(1):13-.9
15. Bayrak GD, Gurdogan-Guler EB, Yildirim Y, Ozturk D, Selvi-Kuvvetli S. Assessment of shear bond strength and microleakage of fissure sealant following enamel deproteinization: An in vitro study. J clinical and experimental dent. 2020; 12(3):e220-e226.
16. Di Renzo M, Ellis TH, Sacher E, Stangel I. A photoacoustic FTIRS study of the chemical modifications of human dentin surfaces: II. Deproteination. Biomaterials. 2001; 22(8):793-7.
17. Hu X, Peng Y, Sum CP, Ling J. Effects of concentrations and exposure times of sodium hypochlorite on dentin deproteination: attenuated total reflection Fourier transform infrared spectroscopy study. J endod. 2010; 36(12):2008-11.
18. Ekambaram M, Anthonappa RP, Govindool SR, Yiu CKY. Comparison of deproteinization agents on bonding to developmentally hypomineralized enamel. Journal of dentistry. 2017; 67:94-101.
19. Huilcapi M, Armas-Vega A, Cardenas AFM, et al. Effect of surface treatments on the adhesive properties of metallic brackets on fluorotic enamel. Dental press j orthodont. 2020; 25(4):59-6.7
20. Wright JT, Chen SC, Hall KI, Yamauchi M, Bawden JW. Protein characterization of fluorosed human enamel. J dent res. 1996; 75(12):1936-41.
21. Ahmed AM, Nagy D, Elkateb MA. Etching Patterns of Sodium Hypochlorite Pretreated Hypocalcified Amelogenesis Imperfecta Primary Molars: SEM Study. J clin ped dent. 2019 43(4):257-262.
22. Loguercio AD, Muñoz MA, Luque-Martinez I, Hass V, Reis A, Perdigão J. Does active application of universal adhesives to enamel in self-etch mode improve their performance? Journal of dentistry. 2015; 43(9):1060-1070.
23. Huang X, Xie J, Lan Y, Sun Z, Zhang M, Guo L. The effects of 45S5 bioactive glass and Er:YAG Laser on the microtensile bond strength of fluorosed teeth. Microscopy research and technique. 2020; 83(12):1558-1565.
24. Kim IH, Son JS, Min BK, Kim YK, Kim KH, Kwon TY. A simple, sensitive and non-destructive technique for characterizing bovine dental enamel erosion: attenuated total reflection Fourier transform infrared spectroscopy. International journal of oral science. 2016; 8(1):54-60.
25. Sun L, Liang S, Sa Y, et al. Surface alteration of human tooth enamel subjected to acidic and neutral 30% hydrogen peroxide. J dent. 2011; 39(10):686-92.
26. Bistey T, Nagy IP, Simó A, Hegedus C. In vitro FT-IR study of the effects of hydrogen peroxide on superficial tooth enamel. J dent. 200735(4):325-30.
27. Jiang T, Ma X, Wang Y, Zhu Z, Tong H, Hu J. Effects of hydrogen peroxide on human dentin structure. Journal of dental research. 2007; 86(11):1040-5
28. Sa Y, Jiang T, Li BY, Wang ZJ, Wang ZH, Wang YN. [Effects of three at-home bleaching agents on enamel structure and structure-related mechanical properties]. Zhonghua kou qiang yi xue za zhi = Zhonghua kouqiang yixue zazhi = Chinese journal of stomatology. 2012; 47(5):281-6.
29. Sa Y, Liang S, Ma X, et al. Compositional, structural and mechanical comparisons of normal enamel and hypomaturation enamel. Acta biomaterialia. 2014; 10(12):5169-5177.
30. Valencia R, Espinosa R, Borovoy N, Pérez S, Ceja I, Saadia M. Deproteinization Effectiveness on Occlusal Enamel Surfaces and Resultant Acid Etching Patterns: An in vitro Study. J clin pediat dent. 2018; 42(6):434-441.
31. Abdelmegid FY. Effect of deproteinization before and after acid etching on the surface roughness of immature permanent enamel. Nigerian journal of clinical practice.2018; 21(5):591-596.
32. Weston CH, Ito S, Wadgaonkar B, Pashley DH. Effects of time and concentration of sodium ascorbate on reversal of NaOCl-induced reduction in bond strengths. J endod. 2007; 33(7):879-81.
33. DenBesten P, Li W. Chronic fluoride toxicity: dental fluorosis. Monographs in oral science 2011;. 22:81-96.