투고일 : 2020. 1. 28 심사일 : 2020. 2. 17 게재확정일 : 2020. 2. 25

1

Effect of fluoride varnishes on the surface hardness of bovine teeth under demineralization/remineralization cycling

[1]Department of Dental Biomaterials, College of Dentistry, Wonkwang University
[2]Kyungpook national university dental hospital
[3]College of Dentistry, Wonkwang University
[4]Department of Dental Biomaterials and Institute of Biomaterials-Implant, College of Dentistry, Wonkwang University

Ju-Lee Son[1], Yoon-Jeong Shin[2], Geon-Hee Jeong[3], Shin-Jae Choi[3], Seunghan Oh[4], Ji-Myung Bae[4]

ABSTRACT

Effect of fluoride varnishes on the surface hardness of bovine teeth under demineralization/remineralization cycling

[1]Department of Dental Biomaterials, College of Dentistry, Wonkwang University
[2]Kyungpook national university dental hospital
[3]College of Dentistry, Wonkwang University
[4]Department of Dental Biomaterials and Institute of Biomaterials-Implant, College of Dentistry, Wonkwang University

Ju-Lee Son[1], Yoon-Jeong Shin[2], Geon-Hee Jeong[3], Shin-Jae Choi[3], Seunghan Oh[4], Ji-Myung Bae[4]

We investigated whether fluoride varnishes recover the hardness of bovine teeth under 20 days of demineralization/remineralization cycling. The fluoride varnish groups (two commercial fluoride varnishes [V-varnish (Vericom, Korea) and CavityShield (3M ESPE, USA)] and an experimental fluoride varnish including 5 wt.% NaF were compared with a control group without fluoride varnish. Vickers hardness was measured at baseline, 3 days after immersion in caries-inducing solution, 24 hours after application of a fluoride varnish, and after 10 and 20 days of demineralization/remineralization cycling. Afterward, tooth surfaces were observed by scanning electron microscope. After fluoride varnish application and the cycling 10 and 20 days, the experimental varnish group showed the highest hardness, while the CavityShield and the control groups demonstrated the lowest hardness. The experimental varnish group recovered the hardness of the baseline at 24 hours after application of the varnish, while it was recovered after 20 days of the cycling in case of the V-varnish. However, the CavityShield and the control groups did not recover the hardness even after 20 days of the cycling. The experimental fluoride varnish with fast recovery in the hardness of the baseline can be used as an effective fluoride varnish to resist demineralization and to facilitate remineralization.

Key words: Fluoride varnish, Vickers hardness, Demineralization/Remineralization cycling

Corresponding Author
Ji-Myung BAE
Department of Dental Biomaterials, College of Dentistry, Wonkwang University 460 Iksan-daero, Iksan city, Jeonbuk 54538, Republic Korea
E-mail: baejimy@wku.ac.kr

ACKNOWLEDGMENT This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (2018R1A2B6002088)

I. Introduction

It is well-known that Streptococcus mutans is the most representative bacterium that causes dental caries[1]. It is also widely accepted that the main mechanism through which fluoride controls dental caries is by the topical effects of low levels of fluoride on the enamel, plaque, and saliva interface[2]. The most common topical fluoride agents include fluoride gel, fluoride foam, fluoride mouth rinse, toothpaste, and fluoride varnish[3-5]. Among fluoride agents, fluoride varnish exhibits the highest inhibitory effects against dental caries (38%); thus, it has become the most common fluoride agent of choice[6]. Fluoride varnish typically contains 5% sodium fluoride (NaF)[7]. Because of its characteristic viscosity, it attaches to the tooth surface and releases fluoride for an extended duration, thereby serving as a slow-release fluoride reservoir[8-10]. Among various fluoride agents, fluoride varnish is the easiest to manipulate and the least dependent on patient cooperation[11]. It can be used as a very effective dental caries preventive agent for patients with high risks of developing dental caries[8]. In addition, it can be used to prevent root caries in the older population[12].

Calcium fluoride (CaF_2) is formed on the enamel surface and in subsurface carious lesions when the fluoride concentration is high; fluorapatite is deposited when the fluoride concentration is low. CaF_2 precipitates are easily removed in alkaline solutions, while fluorapatite is permanently bound within the enamel crystal structure[3,13,14]. In specific conditions, components of CaF_2 may be redeposited as fluorapatite, and this transformation is accelerated in the presence of fluoride varnish[3]. The structure of fluorapatite is more resistant to caries than that of hydroxyapatite[2]. Indeed, fluoride ions of fluoride varnish diffuse into carious lesions and reduce the porosity of the lesions[15]. When a low, sustained concentration (sub-ppm) of fluoride is present in the oral fluid, it prevents demineralization during acidic challenge by becoming adsorbed to hydroxyapatite crystals; additionally, it accelerates the remineralization process when pH increases above [5,52]. The usefulness of fluoride-releasing agents for the remineralization of enamel has been shown in multiple studies[16,17].

According to caries-related studies, changes in the microhardness of dentin are directly related to its mineral content[18-21]. Measuring the hardness of a tooth is a reasonable method of examining its mineral content[21,22]. Therefore, we assessed the degree of remineralization of the bovine tooth surface by measuring the Vickers hardness in various test conditions.

Although there have been studies showing that fluoride varnish affects mineral loss or remineralization under conditions of demineralization/remineralization cycling[23,24], few studies have investigated its effects on the hardness of teeth. In most related studies, hardness was measured after a short period of approximately 7 days[25,26]; no study has compared long-term effects according to the type of fluoride varnish, including experimental fluoride varnish.

The purpose of this study was to examine how effectively fluoride varnish prevented demineralization and facilitated remineralization by measuring the hardness of bovine teeth. Three experimental groups, including two kinds of conventional fluoride varnish products and one experimental fluoride varnish, were divided. The control group comprised bovine teeth without a fluoride varnish. After inducing caries, fluoride varnish was applied and demineralization/remineralization cycling was performed for 20 days. The null hypothesis for this study was that no significant differences would be observed among the three experimental groups and the control group by fluoride varnish application and demineralization/remineralization cycling.

II. Materials and Methods

1. Specimen preparation

Forty clean bovine incisors without caries were prepared, and 10 incisors were allocated to each group for hardness testing. An additional three incisors were allocated to each group for scanning electron microscope (SEM) observation and Energy Dispersive X-ray spectroscopy (EDS) analysis. A 10x7-mm tape was placed on the labial surface of each tooth after cutting the root and removing the pulp, and nail varnish was applied in the remaining areas of the surface (Fig. 1A). After the nail varnish had dried, the bovine incisors were embedded in acrylic resin (Ortho-Jet, Lang Dental, USA) and the tape was removed to form a window (Fig. 1B). The specimens were then immersed in distilled water in a sealed container and placed in a dry oven (FO-600M, JEIO TECH, Korea) at 37°C for 24 hours, after which the Vickers hardness was measured. The overall experimental procedure is

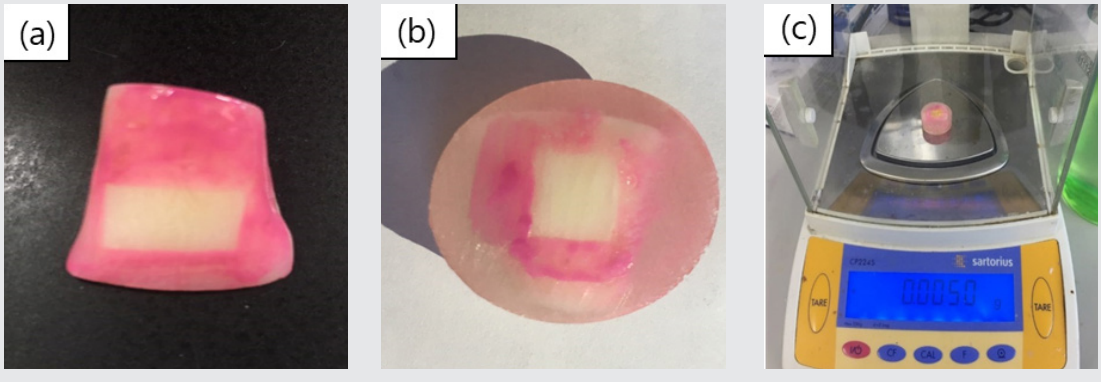

Fig. 1. Preparation of specimens with bovine teeth. A. Application of nail varnish on the tooth surface surrounding the tape. B. Embedding of the tooth in acrylic resin and formation of a window by removing the tape. C. Application of fluoride varnish on the window until a weight of 5 mg of varnish was measured on a balance.

illustrated in Fig. 2.

2. Caries-inducing solution

A caries-inducing solution (acidic solution) was prepared with 8.7 mmol/L $CaCl_2$, 8.7 mmol/L KH_2PO_4, 0.05 ppm F from NaF, and 75 mmol/L acetic acid (pH=4.0)[24]. Hardness was measured after storing the embedded teeth in the solution at 37°C in a shaking water bath at 100 rpm (JSSI-100C, JS Research Inc., Korea) for 3 days.

3. Fluoride varnish application

An experimental fluoride varnish (EX) and two existing fluoride varnish products were used: V-varnishTM (VV, Vericom, Korea), and CavityShieldTM (CS, 3M ESPE, USA). The EX varnish was prepared with 5 wt.% NaF (Sigma), 50 wt.% ethanol (absolute≥99.7%, Merck) as a solvent, and 45 wt.% rosin base (KR-610, Arakawa Chemical Industries Ltd., Japan) as a base[27-29].

Five milligrams of fluoride varnish were applied

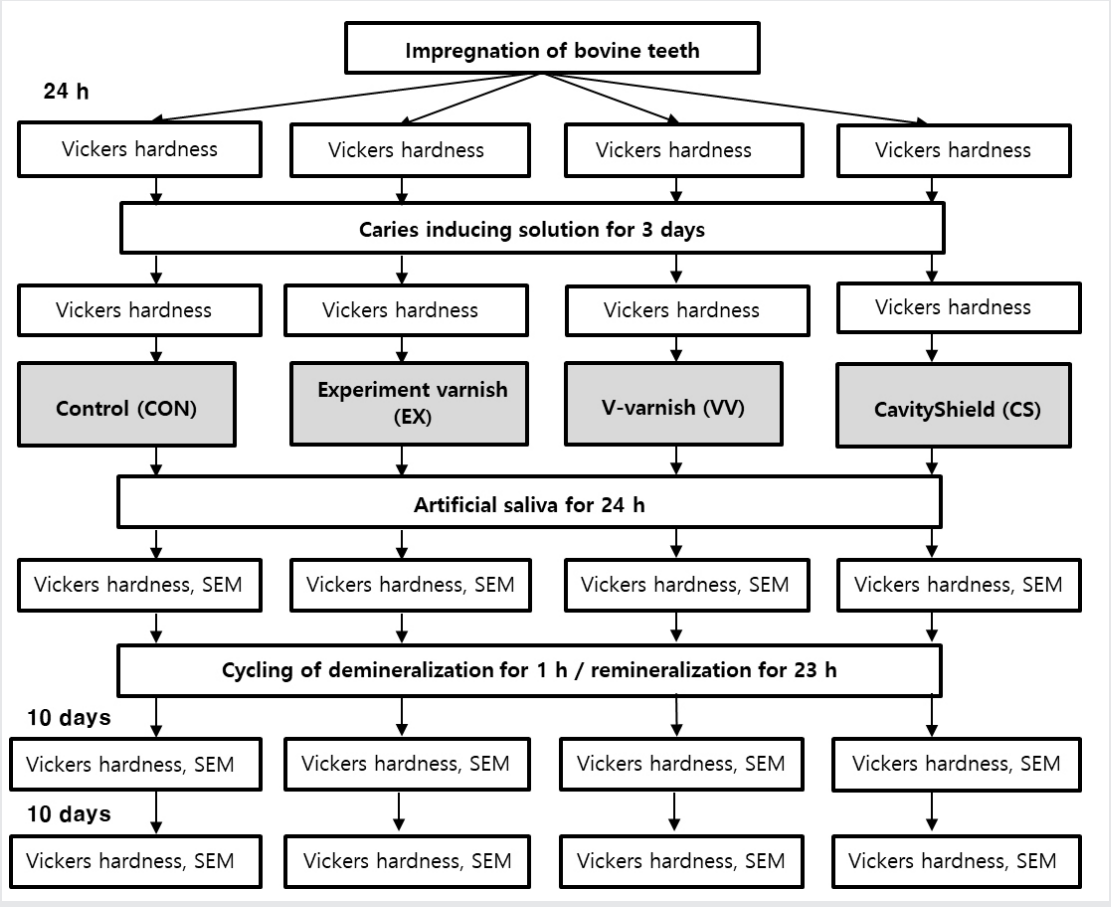

Fig. 2. Schematic diagram of the experimental design. SEM means scanning electron microscopic examination.

to the bovine incisor window, using a scale (CP224S, Satorius) for measurement (Fig. 1C). For the control group (CON), the bovine incisors were left unvarnished.

Artificial saliva was fabricated with 0.4g NaCl, 0.4 g KCl, 0.795 g $CaCl_2 \cdot 2H_2O$, 0.78 g $NaH_2PO4 \cdot 2H_2O$, 0.005 g $Na_2S \cdot 9H_2O$, and 1.0 g NH_2CONH_2 in 1000 mL distilled water (pH=7)[30]. After applying a fluoride varnish, the bovine teeth were stored in an artificial saliva in a shaking water bath at 37°C and 100 rpm for 24 hours before measuring hardness.

4. Demineralization/remineralization cycling

Demineralization solution was prepared with 2.0 mmol/L $CaCl_2$, 2.0 mmol/L KH_2PO_4, and 75 mmol/L acetic acid (pH=4.3)[23]. Remineralization solution was prepared with 1.2 mmol/L $CaCl_2$, 0.72 mmol/L K_2HPO_4, 2.6 mol/L F, and 50 mmol/L HEPES buffer (pH=7.0)[24].

The embedded bovine teeth were stored in the demineralization solution for 1 hour and then transferred to the remineralization solution for 23 hours in a shaking water bath at 37°C and 100 rpm. The solution was changed daily. Hardness was measured on days 10 and days 20 of cycling.

5. Vickers hardness test

As shown in Fig. 2, hardness was measured 24 hours after embedding the teeth in an acrylic resin as a baseline, 3 days after storing in an acidic solution, 24 hours after applying fluoride varnish, and after 10 days and 20 days of demineralization/remineralization cycling. The window surface of the bovine teeth was indented for 20 seconds at 400 gf by using a Vickers hardness tester (AVK-C1, Akashi Co., Japan), and the diagonal length of the indentation was measured. Three indents were created for each measurement, and the mean value was used. The Vickers hardness value was computed by using the following equation:

$$HV = 1.854 \times P/d2$$

P: the load applied (kg)
d: the length of the average diagonal of the indentation (mm)

6. SEM observation and EDS analysis

After the fluoride varnish application, and 10 and 20 days of demineralization/remineralization cycling, three specimens from each group were observed by using a SEM (JSM-6360, Jeol Ltd., Japan) at ×500. In addition, after 20 days of demineralization/remineralization cycling, changes of tooth surface composition were measured by using Energy Dispersive X-ray spectroscopy (EDS, Oxford Instruments Analytical 7582, UK).

7. Statistical analysis

Statistical analysis was performed by using SPSS software (SPSS 22.0; SPSS GmbH, Munich, Germany). The Kruskal-Wallis test was used at a confidence interval of 95%, and Tukey's HSD test was used for post hoc testing.

Table 1. Surface microhardness values of bovine teeth, with statistical rankings within each measurement and within each group

Measurement	Treatment	CON	EX	VV	CS
1st	Baseline	236.6^{Aa} (25.5)	220.6^{Ab} (30.1)	222.5^{Ab} (16.2)	235.2^{Aa} (29.7)
2nd	Caries-inducing	38.4^{Ac} (10.8)	41.8^{Ac} (3.9)	39.3^{Ad} (9.1)	37.4^{Ad} (3.9)
3rd	*F varnish 24 h	55.1^{Cc} (32.2)	186.0^{Ab} (47.8)	126.1^{Bc} (22.6)	46.2^{Cd} (7.9)
4th	†Cycling 10 d	67.0^{Cc} (18.4)	231.2^{Ab} (38.9)	163.0^{Bc} (31)	82.1^{Cc} (29.4)
5th	†Cycling 20 d	102.5^{Cb} (31.9)	418.8^{Aa} (81.9)	293.4^{Ba} (44.5)	127.9^{Cb} (30.2)

Results are shown as mean and SD in the parenthesis. Different uppercase letters indicate significant differences among the groups at the same measurement (row); different lowercase letters indicate significant differences among measurements within each group (column) by Kruskal–Wallis and Tukey HSD test at a = 0.05. *F varnish means fluoride varnish. †Cycling denotes demineralization for 1 hour and remineralization for 23 hours. CON: control; EX: experimental varnish; VV: V-varnish; CS: CavityShield

Table 2. Element analysis of tooth surfaces by using energy dispersive X-ray spectroscopy (EDS), with values expressed as mean (SD)

Element	CON	EX	VV	CS
C	0.94 (0.06)	41.63 (2.24)	45.15 (1.50)	2.01 (1.32)
O	26.83 (0.39)	37.59 (14.78)	25.75 (2.88)	28.38 (1.67)
Ca	30.42 (1.02)	10.01 (0.78)	5.26 (0.85)	29.90 (2.19)

Each value represents the percentage of the whole constituted by the given element. CON: control; EX: experimental varnish; VV: V-varnish; CS: CavityShield

Ⅲ. Results

1.Vickers hardness

The graphs of Vickers hardness for the three fluoride varnish groups and control group are shown in Fig. 3. The data, including statistical rankings, among groups within each treatment and according to treatments within each group, are shown in Table 1. There were no significant differences in hardness among the groups at baseline ($p>0.05$). After caries inducing, the hardness of all groups decreased significantly from the baseline ($p<0.05$) with no significant differences among the groups ($p>0.05$). However, after 24 hours' storage in artificial saliva since fluoride varnish was applied, only EX group recovered the hardness of the baseline and showed the highest hardness among the groups ($p<0.05$). After 10 and 20 days of the demineralization/remineralization cycling, the hardness of the EX group was the highest, while the CS and the control group showed the lowest hardness ($p<0.05$). After 20 days of the demineralization/remineralization cycling,

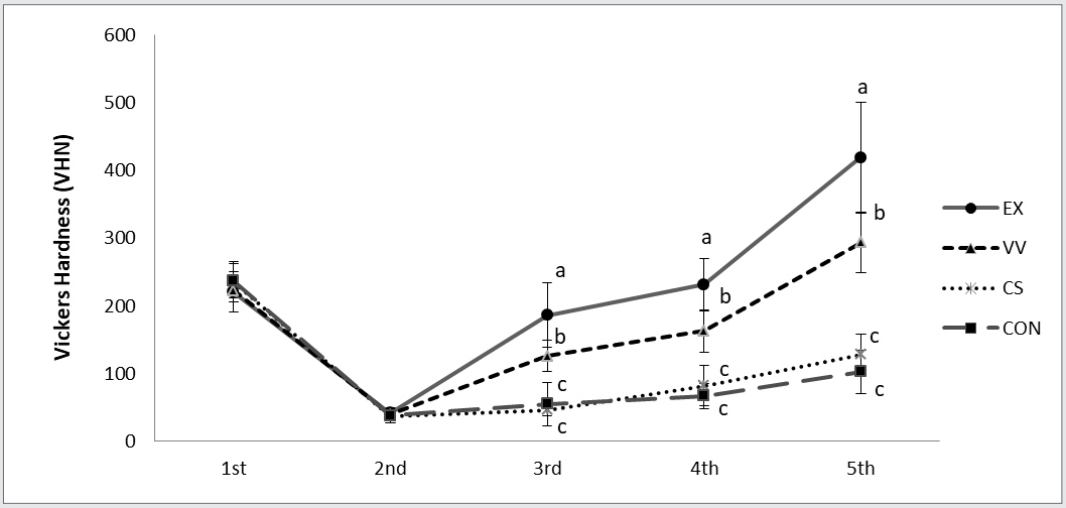

Fig. 3. Surface microhardness of each group at different measurement times. Different lowercase letters indicate significant differences among groups within each measurement by Kruskal–Wallis and Tukey HSD at α=0.05. CON: control; EX: experimental varnish; VV: V-varnish; CS: CavityShield

the hardness of the EX and VV groups were even higher than those of the baseline ($p<0.05$). However, the CS and the control group did not recover the hardness of the baseline even after 20 days of the cycling.

2. SEM observation

Fig. 4 shows SEM observations of the tooth surface of all groups after fluoride varnish application followed by 24 hours of storage in artificial saliva, and after 10 and 20 days of demineralization/remineralization cycling. It is apparent that the demineralized subsurface enamel is covered with fluoride varnish after storage in artificial saliva for 24 hours, for all experimental groups except for the control group. In particular, the EX group shows crystal-like structures on the tooth surface at 24 hours after fluoride varnish application. After 10 days of demineralization/remineralization cycling, the control group showed severe demineralization with exposed enamel rods, while the EX group showed good coverage with fluoride varnish. The fluoride varnish was slightly removed in the VV group and substantially removed in the CS group. After 20 days of demineralization/remineralization cycling, partial remineralization had occurred in the control group, resulting in substantial reduction in etched surfaces. While the fluoride varnish was well-preserved in the EX group, a considerable portion of the varnish was removed in the CS group.

3. EDS analysis

Table 2 shows the results of EDS analysis of the

bovine tooth surface after 20 days of demineralization/remineralization cycling. Fluoride was not detected in any group. The percentage of carbon (C) was higher, while that of calcium (Ca) was lower in the EX and VV groups than in the control and CS groups.

Ⅳ. Discussion

This study aimed to evaluate how effectively fluoride varnish prevented demineralization and facilitated remineralization during demineralization/remineralization cycling by measuring the hardness of teeth. We induced artificial caries in bovine teeth by submerging them in a caries-inducing solution, followed by application of fluoride varnish, and demineralization/remineralization cycling. Tooth hardness was measured to evaluate the degree to which demineralization was inhibited and remineralization was facilitated by the fluoride varnish. The baseline hardness was equal for all groups, and after caries-inducing treatment, there were no significant differences in hardness among the groups;

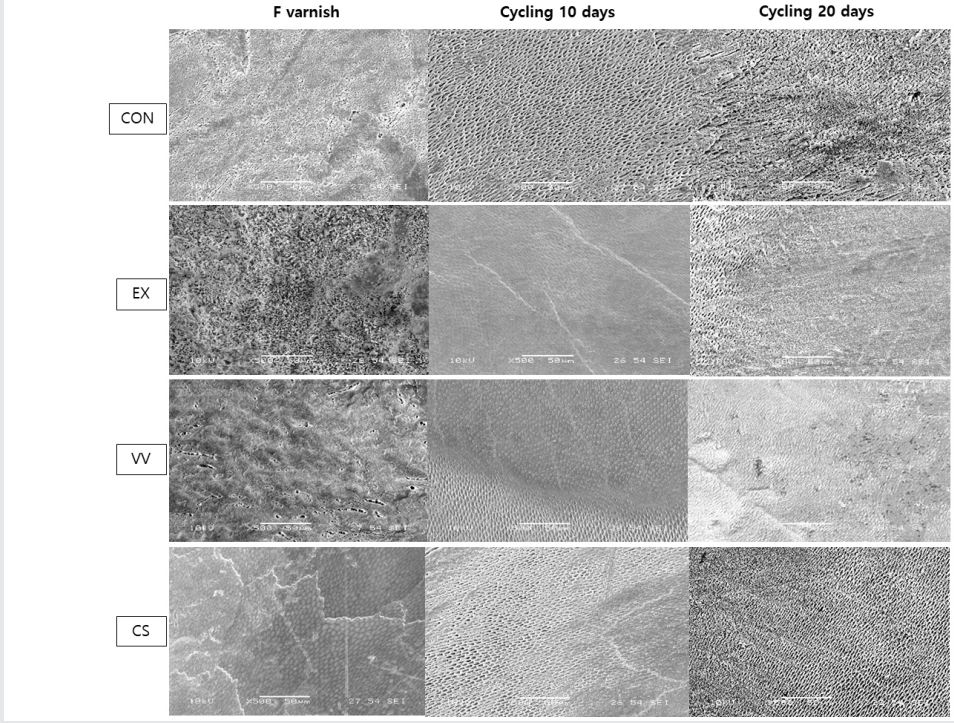

Fig. 2. Schematic diagram of the experimental design. SEM means scanning electron microscopic examination.

thus, there were no differences in hardness prior to application of the fluoride varnishes.

Twenty-four hours after applying the fluoride varnishes, the hardness was the highest in the EX group, followed by VV and CS groups. Notably, the remineralization rate was sufficiently high in the EX group that recovered the hardness of the baseline only at 24 hours after applying fluoride varnish. The fluoride release of higher amounts initially and for longer period of the EX compared with the VV and CS, can be considered to attribute to the facilitated remineralization of the EX group[27]. The hardness in the CS group did not significantly differ from that in the control group. The same pattern of results was observed after 10 and 20 days of demineralization/remineralization cycling. Therefore, the null hypothesis was rejected.

The EX group recovered to baseline hardness after 24 hours of application of the fluoride varnish. Further, the hardness of EX and VV groups increased to levels beyond baseline after 20 days of demineralization/remineralization cycling. Previous studies involving demineralization/remineralization cycling only adopted 7-day cycles[25,26]; importantly, the hardness of teeth did not recover to baseline in that period. Therefore, based on our findings, a cycle of 20 days or longer is recommended to evaluate remineralization effects.

After demineralization/remineralization cycling, enamel crystals differ from their original state[31,32]. Furthermore, calcium and phosphate ions are essential, in addition to fluoride ions, in order to accelerate remineralization and form fluorapatite[33]. The remineralization solution used in this study contained calcium and phosphate ions, and we replaced the solution daily; thus, extended demineralization/remineralization cycling resulted in increased remineralization. Reynolds et al. also reported that elevation of Ca and P concentrations in tooth enamel accelerates remineralization, strengthening the tooth structure[34].

As confirmed by the SEM results, the experimental varnish adhered better to the tooth surface after the application of fluoride varnish and during the demineralization/remineralization cycling, implying the release of fluoride for a longer period. Indeed, a higher quantity of fluoride varnish remnants on the tooth surface in SEM observations was associated with higher hardness, suggesting a proportional relationship between the two. As explained by Buzalaf et al.[2], fluorapatite formation occurs by nucleation of partially dissolved minerals on tooth surfaces containing fluoride and less carbonate, rendering the enamel more resistant to future acidic challenges. A low level of fluoride present for prolonged periods is adsorbed to the partially demineralized crystal surface and attracts calcium ions[2], thereby contributing to accelerated remineralization and increased hardness of teeth.

In EDS analysis of the tooth surface after 20 days of demineralization/remineralization cycling, fluoride was not detected in any of the groups. The EX and VV groups showed increased carbon content, but reduced Ca content, compared with the control and CS groups. This may be a result of the fluoride varnish covering a substantial portion of

the enamel in the EX and VV groups—carbon is the major component of rosin, the base of fluoride varnish. In contrast, the calcium content was increased in the control group, which was not treated with fluoride varnish, and in the CS group, where the fluoride varnish was almost entirely removed. Because it seems difficult to measure trace amounts of fluoride on tooth surfaces with EDS, XRD may be necessary to detect the small quantity of ions on the tooth surface.

Bovine teeth were selected in the study because of the similarity in the hardness and the chemical composition[35], and the affordability. Further studies should quantify the exact amounts of fluoride released into the artificial saliva and demineralization/remineralization solution daily. A previous study reported that the addition of casein phosphopeptide-stabilized amorphous calcium phosphate complexes (CPP-ACP) to fluoride varnish could remineralize artificial dentin caries-like lesions under demineralizing conditions[36]. Subsequent studies should assess whether the addition of CPP-ACP or calcium and phosphate to experimental fluoride varnish increases tooth remineralization.

Under the conditions of this study, at least 20 days of demineralization/remineralization cycling were required to fully assess the effects of remineralization. Fluoride varnishes should be chosen with discretion because the rate of remineralization after the demineralization/remineralization cycling differed according to the type of fluoride varnish used. The fast recovery of the hardness in the experimental fluoride varnish group suggests that it can be used as an effective to resist dental caries and to facilitate remineralization.

V. Conclusion

When fluoride varnish was applied on the tooth surface after caries induction, the EX group recovered the hardness of the baseline in 24 hours' storage in artificial saliva. After 20 days' demineralization/remineralization cycling, the hardness of the EX and VV group even increased than those of the baseline. The experimental fluoride varnish with fast recovery in the hardness of the baseline can be used as an effective fluoride varnish to resist demineralization and to facilitate remineralization, suggesting the possibility of prevention of dental caries.

References

1. Tanzer JM, Livingston J, Thompson AM. The microbiology of primary dental caries in humans. J Dent Educ 2001; 65: 1028–1037
2. Buzalaf M, Pessan J, Honorio H, ten Cate J. Mechanisms of action of fluoride for caries control. Monogr Oral Sci 2011; 22: 97–114
3. Beltrán-Aguilar ED, Goldstein JW, Lockwood SA. Fluoride varnishes: a review of their clinical use, cariostatic mechanism, efficacy and safety. J Am Dent Assoc 2000; 131: 589–596
4. Baysan A, Lynch E, Ellwood R, Davies R, Petersson L, Borsboom P. Reversal of primary root caries using dentifrices containing 5,000 and 1,100 ppm fluoride. Caries Res 2001; 35: 41–46
5. Twetman S, Keller MK. Fluoride rinses, gels and foams: An update of controlled clinical trials. Caries Res 2016; 50: 38–44
6. Newbrun E. Finn Brudevold: discovery of acidulated phosphate fluoride in caries prevention. J Dent Res 2011; 90: 977–980
7. Marinho VC, Worthington HV, Walsh T, Clarkson JE. Fluoride varnishes for preventing dental caries in children and adolescents. Cochrane Database Syst Rev 2013; 7: CD002279
8. Øgaard B, Seppa L, Rolla G. Professional topical fluoride applications - clinical efficacy and mechanism of action. Adv Dent Res 1994; 8: 190–201
9. Weintraub JA, Ramos-Gomez F, Jue B, Shain S, Hoover CI, Featherstone JD, Gansky SA. Fluoride varnish efficacy in preventing early childhood caries. J Dent Res 2006; 85: 172–176
10. Delbem AC, Brighenti FL, Oliveira FA, Pessan JP, Buzalaf MA, Sassaki KT. In vitro assessment of an experimental coat applied over fluoride varnishes. J Appl Oral Sci 2009; 17: 280–283
11. Bravo M, Garcia-Anllo I, Baca P, Llodra JC. A 48-month survival analysis comparing sealant (Delton) with fluoride varnish (Duraphat) in 6- to 8-year-old children. Community Dent Oral Epidemiol 1997; 25: 247–250
12. Tan HP, Lo EC, Dyson JE, Luo Y, Corbet EF. A randomized trial on root caries prevention in elders. J Dent Res 2010; 10: 1086–1090
13. Arends J, Schuthof J. Fluoride content in human enamel after fluoride application and washing: an in vitro study. Caries Res 1975; 9: 363–372
14. Retief DH, Bradley EL, Holbrook M, Switzer P. Enamel fluoride uptake, distribution and retention from topical fluoride agents. Caries Res 1983; 17: 44–51
15. Holmen L, Ögaard B, Rölla G, Thylstrup A. A polarized light and scanning electron microscope study of the effect of Duraphat treatment on in vivo caries. Scand J Dent Res 1986; 94: 521–529
16. Burke FM, Ray NJ, McConnell RJ. Fluoride-containing restorative materials. Int Dent J 2006; 56: 33–43
17. Rodrigues E, Delbem AC, Pedrini D, de Oliveira MS. PH-cycling model to verify the efficacy of fluoride-releasing materials in enamel demineralization. Oper Dent 2008; 33: 658–665
18. Pereira PN, Inokoshi S, Yamada T, Tagami J. Microhardness of in vitro caries inhibition zone adjacent to conventional and resin-modified glass ionomer cements. Dent Mater 1998; 14: 179–185
19. Banerjee A, Sherriff M, Kidd EA, Watson TF. A confocal microscopic study relating the autofluorescence of carious dentine to its microhardness. Br Dent J 1999; 187: 206–210
20. Hosoya Y, Marshall SJ, Watanabe LG, Marshall GW. Microhardness of carious deciduous dentin. Oper Dent 2000; 25: 81–89
21. Chu CH, Lo EC. Microhardness of dentine in primary teeth after topical fluoride applications. J Dent 2008; 36: 387–391
22. Angker L, Nockolds C, Swain MV, Kilpatrick N. Correlating the mechanical properties to the mineral content of carious dentine—a comparative study using an ultra-micro indentation system (UMIS) and SEM-BSE signals. Arch Oral Biol 2004; 49: 369–378
23. Takagi S, Liao H, Chow LC. Effect of a low-fluoride-content, two-component rinse on fluoride uptake and on de-—and remineralisation of enamel lesions: an in vitro study. Caries Res 2006; 35: 223–228
24. Weir MD, Chow LC, Xu HH: Remineralisation of demineralized enamel via calcium phosphate nanocomposite. J Dent Res 2012; 91: 979–1063
25. Kim MJ, Lee SH, Lee NY, Lee IH: Evaluation of the effect of PVA tape supplemented with 2.26% fluoride on enamel demineralization using microhardness assessment and scanning electron microscopy: in vitro study. Arch Oral Biol 2013; 58: 160–166
26. Cardoso CA, Cassiano LP, Costa EN, Souza-E-Silva CM, Magalhães AC, Grizzo LT, Caldana ML, Bastos JR, Buzalaf MA. Effect of xylitol varnishes on remineralisation of artificial enamel caries lesions in situ. J Dent 2016; 50: 74–78
27. Kim AJ: Development and characterization of fluoride varnish with long-term sustained fluoride release and antibacterial activity. Graduate school Wonkwang Universtiy 2015.
28. Shin KS, Kim AJ, Oh SH, Bae JM. Development of fluoride varnish with sustained fluoride release and biocompatibility. Kor J Dent Mater 2017; 44: 21–31
29. Son JL, Kim AJ, Oh SH, Bae JM. Minimum inhibitory concentration and minimum bactericidal concentration of antibacterial fluoride varnish. Kor J Dent Mater 2018; 45: 139–146
30. Iijima M, Hashimoto M, Kohda N, Nakagaki S, Muguruma T, Endo K, Mizoguchi I. Crystal growth on bioactive glass sputter-coated alumina in artificial saliva. Dent Mater J 2013; 32: 775–780
31. ten Cate JM, Larsen MJ, Pearce EIF, Fejerskov O. Chemical interactions between the tooth and oral fluids. In: Fejerskov O, Kidd E, editors. Dental Caries the Disease and Its Clinical Management. Oxford: Blackwell Munksgaard; 2008. p. 209–231.
32. Featherstone JD. Prevention and reversal of dental caries: role of low level fluoride. Community Dent Oral Epidemiol 1999; 27: 31–40
33. Ekambaram M, Mohd Said SNB, Yiu CKY. A review of enamel remineralisation potential of calcium- and phosphate-based remineralisation systems. Oral Health Prev Dent 2017; 15: 415–420
34. Reynolds EC, Cai F, Shen P, Walker GD. Retention in plaque and remineralization of enamel lesions by various forms of calcium in

References

a mouthrinse or sugarfree chewing gum. J Dent Res 2003; 82: 206-211
35. Fonseca RB, Haiter-Neto F, Carlo HL, Soares CJ, Sinhoreti MA, Puppin-Rontani RM, Correr-Sobrinho L. Radiodensity and hardness of enamel and dentin of human and bovine teeth, varying bovine teeth age. Arch Oral Biol 2008; 53: 1023-1029
36. Wierichs RJ, Stausberg S, Lausch J, Meyer-Lueckel H, Esteves-Oliveira M. Caries-preventive effect of NaF, NaF plus TCP, NaF plus CPP-ACP, and SDF varnishes on sound dentin and artificial dentin caries in vitro. Caries Res 2018; 52: 199-211