Remineralization effects when using different methods to apply fluoride varnish \textit{in vitro}

Han-Na Kim\textsuperscript{a}, Jin-Bom Kim\textsuperscript{b,c}, Seung-Hwa Jeong\textsuperscript{b,c}\textsuperscript{*}

\textsuperscript{a} Department of Dental Hygiene, College of Health and Medical Sciences, Cheongju University, Daesung-ro 298, Cheongwon-gu, Cheongju, 28503, South Korea
\textsuperscript{b} Department of Preventive and Community Dentistry, School of Dentistry, Pusan National University, Busandaehak-ro 49, Mulgeum-eup, Yangsan-si, Gyeongsangnam-do, 50612, South Korea
\textsuperscript{c} BK PLUS Project, School of Dentistry, Pusan National University, Yangsan, South Korea

Received 8 February 2018; Final revision received 12 May 2018
Available online 1 September 2018

\textbf{KEYWORDS}
Artificial caries lesion;
Demineralization;
Enamel;
Fluoride varnish;
Remineralization;
Vickers hardness number

\textbf{Abstract} Background/purpose: Remineralization efficacy for early caries lesion may change when fluoride varnish (FV) is applied directly or indirectly to the lesion. This \textit{in vitro} study compared direct and indirect remineralization efficacies of FV on artificial caries lesions and evaluated acid-resistance of lesion remineralized by FV and artificial saliva.

\textit{Materials and methods:} One hundred and twenty-six bovine demineralized specimens were allocated to four varnish groups (Duraphat\textsuperscript{\textregistered}/C226, EnamelPro\textsuperscript{\textregistered}/C226, Mi\textsuperscript{\textregistered}/C228, and ClinproWhite\textsuperscript{\textregistered}/C228, each) and a negative-control group (each). Half of specimens from each varnish group had the FV applied and the other specimens didn’t. The specimens treated and not treated with the FV were immersed carefully from the specimen, and immersion process was continued in fresh artificial saliva for 48 h. The negative-control group was immersed in artificial saliva for same time as in varnish groups. The acid resistance of remineralized specimens from varnish groups was compared to negative-control group. Vickers microhardness number (VHN) was measured to evaluate re-demineralization effect.

\textbf{Results:} The $\Delta$VHN was significantly higher for indirect remineralization ($134.4 \pm 31.5$, mean $\pm$ SD) than for direct remineralization ($66.8 \pm 27.9$). All varnish groups showed significant differences between the direct and indirect application methods. The acid resistance of remineralized specimens was higher in the all FV groups than in the negative-control.

\textbf{Conclusion:} This \textit{in vitro} study confirmed that the remineralization effect of fluoride varnishes would be higher in the vicinity than the underneath of the varnish treated surface.

\textsuperscript{*} Corresponding author. Department of Preventive and Community Dentistry, Pusan National University, School of Dentistry, Busandaehak-ro 49, Mulgeum-eup, Yangsan-si, Gyeongsangnam-do, 50612, South Korea. Fax: +82 51 510 8221.
\textit{E-mail address:} jsh0917pusan.ac.kr (S.-H. Jeong).

https://doi.org/10.1016/j.jds.2018.07.004
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**Introduction**

Fluoride varnish (FV) is applied to teeth to form a thin film that remains on the teeth for a long time, and thereby provides sustained fluoride release.\(^1\) It was first introduced in Europe in 1964, and numerous clinical studies performed over the past 25 years have identified it as a safe and effective method for applying fluoride.\(^2\) FV has the advantage that a low concentration of fluoride is maintained in the oral cavity for 1–7 days after application, depending on the product, and it remains attached to the tooth for a relatively long time.

Several studies have evaluated the prevention of dental caries by FVs. The Cochran review performed by Marinho et al.,\(^3\) found that FV was effective in reducing dental caries in children and adolescents, including in permanent teeth, with permanent reduction rates of 46% and 33%, respectively. White-spot lesion (WSL) has been defined as subsurface enamel porosity produced by carious demineralization. Initial treatments for WSLs should adopt the most conservative approaches, such as applying topical fluoride (e.g., FV).\(^4\)

The method used by clinicians to apply FV in patients with WSL is normally based on the manufacturer’s recommendation. The FV is most commonly applied to the smooth surface of the tooth using an applicator brush. In particular, FV is normally applied directly above the site of dental caries to facilitate remineralization. However, the present authors questioned whether the varnish should be applied either over the surface of initial dental caries or around them so that fluoride ions are supplied but without blocking the contact of the tooth with saliva. The authors hypothesized that applying FV directly to an initial dental caries surface would prevent saliva contact in the oral cavity and thereby block the supply of minerals from the saliva.

Previous studies have evaluated the effect of the direct application of FV to dental caries on surface remineralization.\(^5,6\) They assumed that calcium, phosphorus, and fluoride directly penetrate the surface through microdefect pores on the dentate surface due to caries. Such studies therefore did not take into account the possibility of the absorption of fluoride released from the FV applied adjacent to the tooth surface in the remineralization process. Castellano and Donly\(^7\) compared the effect of applying FV at different locations on remineralization, and suggested that there was no significant difference between directly applying FV above the initial dental caries surface and applying it around the dental caries surface or in an adjacent area. In contrast, Lippert et al.\(^6\) suggested that there was a significant difference between direct application and applying FV in the adjacent area. The discrepancies between these findings indicate the need for further studies comparing the efficacy of remineralization between indirect- and direct-application methods.

Most studies of dental erosion using an acid-challenge test have involved immersing the tooth in an acidic solution or beverage and comparing the degree of corrosion in sound enamel, with the results suggesting that FVs can prevent enamel loss.\(^8\) No research has compared the resistance to acidic solutions or acid challenges after remineralizing a demineralized specimen using FV. It is expected that fluoride-remineralized teeth will have a high content of fluorapatite, and therefore also exhibit greater resistance to acid challenges.

The purpose of the present study was to compare the remineralization effects on demineralized enamel between the direct application of FV (on the initial dental caries surface) and indirect application (around the dental caries surface or in the adjacent area). We also determined whether any variations in the remineralization rate with application method differed between FV products. Finally, we compared the resistance of remineralized enamel to acid using FV and artificial saliva.

**Materials and methods**

**Bovine specimen preparation and lesion formation**

One hundred and twenty-six enamel specimens (3 mm in diameter) were drilled from extracted bovine teeth. Each specimen was mounted on a polyacrylic rod using self-cured direct acrylic resin. Then specimens were sequentially ground and polished using 600-, 1200-, 2000-, 2400-, and 4000-grit abrasive paper, followed by polishing with a 1-μm gamma alumina abrasive suspension. Specimens with Vickers microhardness numbers (VHNs) from 305 to 360 (317.9 ± 27.6, mean ± SD) were used in this study.

The enamel specimens were immersed in 13 mL of demineralizing gels\(^8\) (0.1 M lactic acid, 0.2% Carbopol ETD 2050, and 50% saturated hydroxyapatites [calcium phosphate]; pH 5.0) at 37°C for 24 h. They were then carefully washed under tap water to remove any excess gel. Only specimens with average VHNs of 60–100 were used as demineralized specimens.

**Fluoride varnish applications**

The 126 demineralized specimens were then divided into the four treatment groups (n = 28 each) and a negative-control group (n = 14) according to their hardness values. The following four types of fluoride varnishes containing 5% NaF as the main component were used in this study: (1) Duraphat\(^9\) (DP, Colgate-Palmolive Co., Guildford, Surrey, UK), (2) Enamel Pro\(^10\) (EP, Premier Dental, Plymouth Meeting, PA, USA), (3) MI\(^11\) (MI, GC corp., Tokyo, Japan), and (4) Clinpro\(^12\) (CP, 3M ESPE, ST. Paul, MN, USA). All of the FV products were stored at room temperature for 24 h.
prior to the start of the experiment. The products were packaged in disposable quantities and fully mixed before being applied in accordance with the manufacturers’ recommendations.

The four treatment groups were directly and indirectly treated with each fluoride varnish. Half of specimens from each treatment group had the FV applied (FV-direct group) and the other specimens did not apply FV but later exposed indirectly to fluoride ion released from specimens applied with FV (FV-indirect group). FV (0.01 mL) was applied evenly to the specimens in the FV-direct group. One piece of the varnish-applied specimen and one piece of the unapplied specimen were paired, and both specimens immersed into the same container with 20 mL of artificial saliva at 37°C for 24 h to induce remineralization of the lesion. The artificial saliva was not exchanged during this period. After immersion for 24 h, the FV that had been applied in the FV-direct group was rubbed off using cotton balls soaked in acetone three times. All of the specimens were then washed in distilled water and finally immersed again in artificial saliva for 48 h at 37°C. A pair of specimens from the negative-control group were only immersed in artificial saliva for 24 h and 48 h continuously. After 24 h, the artificial saliva was replaced with fresh artificial saliva as in the treatment groups. The composition of the artificial saliva was as follows: 1.45 mM CaCl₂·2H₂O, 5.40 mM KH₂PO₄, 14.90 mM KCl, 28.40 mM NaCl, and 2.20 g/L mucin; adjusted to pH 6.8 with 5 M NaOH.⁹

The specimens were then rinsed in distilled water and stored in frozen form until the hardness was measured.

### Acid resistance of remineralized specimens

The acid resistance of the remineralized specimens with and without fluoride was evaluated. The specimens with similar VHNs were collected from the FV-indirect group and the negative-control group. The specimens from the FV-direct group were excluded since this group (the mean of VHNremin: 137.5) showed significantly lower VHNs than the FV-indirect (205.3) and the negative-control (190.2) group (Table 1). Several specimens of FV-indirect group and negative-control group were also ruled out since they had VHN of surface less than 170 and more 250 or relatively severe multiple surface crack problem due to indentation load of microhardness tester. Finally, 40 specimens from the FV-indirect group and 10 specimens from the negative-control group were used for evaluation of acid resistance. Citric acid and orange juice have generally been used for acid-resistance tests of tooth enamel.¹⁰ In the present study the specimens were immersed in 13 mL of commercially available orange juice (pH 2.3) (Minute Maid®, Coca-Cola Korea, Seoul, Korea) at 37°C for 2 h.

### Pre and post-treatment lesion analyses

The treatment effect of each group was evaluated by measuring the VHN of enamel surface with a Vickers microhardness tester (Mitutoyo HM-122*, Mitutoyo, Japan). Four indentation were made on each specimen before and after each treatment using a single load of 100 g for 10 s. The average hardness value of the specimens was determined from four indentations. Following measurements of VHN after treatment were indented at the place adjacent to first measured.

The difference (ΔVHN) between VHNremin and VHNacid was calculated to evaluate remineralization effect by fluoride varnish application mode. The difference (ΔVHN) between VHNremin and VHNacid was calculated to evaluate acid resistance of remineralized specimens. The change ratio was calculated as a percentage according to change ratio (%) = (VHNafter−VHNbefore)/VHNbefore × 100.

### Statistical analysis

One-way ANOVA was used to compare the differences in the VHNs of the specimens after remineralization among the FV-direct, FV-indirect groups and negative-control group and the differences in remineralization according to FV products. Duncan’s multiple-comparison method was used for post-hoc analysis. Differences in the VHNs of the specimens after acid challenges between the FV-indirect and negative-control groups were compared using the independent t-test. A value of P ≤ 0.05 was considered to indicate a statistical significance. All of the statistical analysis was performed using the IBM SPSS statistics program (IBM SPSS 21.0, IBM, Armonk, NY, USA).

### Results

VHNremin did not differ significantly between the FV-direct, FV-indirect, and negative-control groups. After 72 h of remineralization, the hardness increased significantly in the FV-indirect and negative-control groups compared to the

| Groups           | N  | VHN_{demin} | VHN_{remin} | ΔVHN | Change (%) |
|------------------|----|-------------|-------------|------|------------|
| FV-direct group  | 56 | 70.6 ± 13.9 | 137.5 ± 33.4 | 66.8 ± 27.9 | 97.0 ± 41.1 |
| FV-indirect group| 56 | 70.9 ± 12.6 | 205.3 ± 32.9 | 134.4 ± 31.5 | 196.1 ± 56.4 |
| Negative-control | 14 | 70.1 ± 8.0  | 190.2 ± 42.6 | 120.1 ± 38.4 | 170.8 ± 53.2 |
| Total            | 126| 70.7 ± 12.7 | 173.5 ± 47.1 | 102.8 ± 44.6 | 149.3 ± 68.5 |

FV = Fluoride varnish.
Data are mean ± SD values.
Change (%) = (VHN_{remin} − VHN_{demin})/VHN_{demin} × 100.

a Different superscript letters indicate significant differences in columns using ANOVA and post-hoc Duncan’s multiple-comparison method.
Table 2 presents the ΔVHNs according to FV application methods and products. ΔVHN was highest for DP (ΔVHN = 88.8 ± 22.3) and MI (ΔVHN = 79.6 ± 26.1) and lowest for EP (ΔVHN = 40.2 ± 18.7) (p < 0.05) in the FV-direct group, while it was highest for EP (ΔVHN = 153.9 ± 22.3) and MI (ΔVHN = 146.1 ± 20.3) and lowest for DP (ΔVHN = 111.6 ± 39.1) (p < 0.05) in the FV-indirect group. The mean remineralization increases for all products were significantly higher in the FV-indirect group than in the FV-direct group (p < 0.001). EP showed the greatest difference in remineralization between the two application methods, being 61.2 ± 33.2% in the FV-direct group and 228.4 ± 45.5% in the FV-indirect group, while DP showed the lowest difference of less than 25% between the two application methods. DP was remineralized more in the FV-direct group. For EP, the remineralization rate was relatively low in the FV-direct group but remained high in the FV-indirect group. In other words, the remineralization rate varied with the type of FV and the application method (p < 0.001).

Table 3 presents the changes in hardness after the acid challenges using orange juice. The mean difference (ΔVHN) after the test was −130.5 ± 21.3 in the negative-control group and −77.8 ± 21.5 in the FV treatment group (p < 0.001). The ΔVHN and the percentage change after the test differed significantly according to the FV product (p < 0.001). The percentage change of MI and EP were −30.4 ± 9.6 and −33.4 ± 9.5, respectively, and were significantly higher for DP (−43.0 ± 10.4) and CP (−43.3 ± 6.8) (both p < 0.05).

Discussion

Many laboratory and clinical research studies have demonstrated the effectiveness of FVs. However, knowledge of the differences in initial carious mineralization according to FV application method is necessary when investigating methods for improving the remineralization efficacy. The purpose of this study was to determine the difference in remineralization between the direct and indirect application of FV on demineralized specimens by measuring changes in the surface micro-hardness in vitro.

This study found that hardness increased more in the FV-direct and negative-control groups than in the FV-direct group after 72 h of remineralization treatment. It is considered that components of FV such as a resin base and rosin inhibited the contact between the artificial saliva and the demineralization specimen during the initial remineralization period, thereby interfering with the infiltration of inorganic ions and fluoride.

It is known that the mechanism underlying remineralization by fluoride ions also promotes the remineralization of a caries lesion. In contrast, this study found no statistically significant difference in the increase of hardness after remineralization between the FV-indirect and negative-control groups. That is, in the present in vitro experimental model, the presence of fluoride in the immersion solution due to the application of FV did not significantly affect the increase in hardness. These results can be attributed to the composition of the artificial saliva used for the remineralization process. The artificial saliva used in many studies to reproduce real oral conditions is rich in minerals, which results in its being effective for remineralization. Artificial saliva has also been used as a remineralization solution in the pH-cycling model. The artificial saliva used in the present experiment was supersaturated with calcium and phosphorus, which can be assumed to underlie why there was no significant difference between the FV-indirect and negative-control groups.

The increase in hardness of the initial caries lesion was greater in the FV-indirect group than in the FV-direct group for all four products tested in this study: the increase was 3.73-fold for EP, 2.02-fold for CP, 1.99-fold for MI, and 1.20-fold for DP. Lippert et al. reported that the hardness of the FV as measured using the Knoop surface microhardness.
test after remineralization treatment increased significantly when the FV was applied indirectly to the adjacent tooth surface compared to applying FV directly to the demineralized tooth surface. That study used four types of FV: CS, EP, MI, and Prevident (Colgate®), and the hardness increase was 1.1- to 2.0-fold higher for indirect application than for direct application, depending on the product. However, those authors focused on remineralization according to the application method of FV and the product used, and they did not compare differences in the remineralization effects with the negative-controls in which specimens were immersed into artificial saliva and an acid-challenge test was performed, which represent substantial generalization effects with the negative-controls in which saliva passed through the FV and acted on the surface of the artificial caries. In the present study, EP and MI showed a significant hardness increase compared to the other FV products. EP and MI exhibited higher efficacy for which the artificial saliva was kept from the specimen through the FV compared to the other products, or the time for which the artificial saliva was kept from the specimen was shorter than that for the other products.

In the FV-indirect group, EP showed a significant hardness increase compared to the other FV products. EP releases fluoride and mineral ions and seems to have a great remineralization effect. MI and EP exhibited higher efficacy when they were applied in the vicinity rather than directly to the lesion, compared to fluoride uptake in the enamel. The significant difference in the increase in VHN depending on the product in the indirect-application group could be due to the difference in the concentration of fluoride dissolved in the solution. A previous study found that the fluoride concentration differed significantly among five FV products, with EP and MI contributing significantly more fluoride ions after 96 h of immersion compared to the other three products. In the present study, EP and MI produced larger hardness increase after remineralization among the FV-indirect group, and the difference in remineralization among those groups may be due to differences in the amount of released fluoride ions.

The presence of fluoride ions is reported to improve the acidic dissolution resistance of the enamel and protect the enamel surface. It is well known that fluorapatite is more resistant to acid than is hydroxyapatite. In the acid-challenge test involving orange juice, the loss of enamel hardness (i.e., ΔVHN) was significantly greater in the control group than in the FV-indirect group. That is, there was no significant difference in remineralization depending on the presence or absence of FV, but there was a significant difference in the loss of hardness in the acid-resistance test. The remineralized specimens in artificial saliva contained hydroxyapatite damaged by demineralization due to the penetration of calcium and phosphorus ions in the

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**Table 3  Differences in enamel hardness after immersion in orange juice for 2 h.**

| Group                    | N  | VHN<sub>emin</sub> (VHN<sub>acid</sub>) | VHN<sub>acid</sub> | ΔVHN | Change (%) |
|--------------------------|----|--------------------------------------|-------------------|------|------------|
| Negative-control group   | 10 | 212.9 ± 19.0                         | 82.5 ± 21.1       | −130.5 ± 21.3 | −61.4 ± 8.9 |
| Fluoride treatment group | 40 | 211.1 ± 22.2                         | 133.3 ± 28.4      | −77.8 ± 21.5  | −37.1 ± 10.6 |
| Fluoride Treatment group | DP | 10 204.8 ± 26.8                      | 117.0 ± 26.3<sup>a</sup> | −87.8 ± 22.5<sup>b,c</sup> | −43.0 ± 10.4<sup>a</sup> |
|                          | EP | 9 213.4 ± 18.4                       | 142.7 ± 27.5<sup>b</sup> | −70.7 ± 17.7<sup>d</sup> | −33.4 ± 9.5<sup>b</sup> |
|                          | MI | 12 216.3 ± 18.8                      | 150.2 ± 23.0<sup>b</sup> | −66.1 ± 22.7<sup>a</sup> | −30.4 ± 9.6<sup>b</sup> |
|                          | CP | 6 209.0 ± 25.8                       | 119.4 ± 24.5<sup>c</sup> | −89.6 ± 11.2<sup>c</sup> | −43.3 ± 6.8<sup>a</sup> |

DP = Duraphat<sup>a</sup>; EP = EnamelPro<sup>a</sup>; MI = MI Varnish<sup>a</sup>; CP = Clinpro<sup>a</sup> White Varnish.
Date are mean ± SD values.
No significant intergroup differences in VHN<sub>emin</sub>.
Change (%) = (VHN<sub>acid</sub> − VHN<sub>emin</sub>) / VHN<sub>emin</sub> × 100.
Independent sample t-test showed significant intergroup difference in VHN<sub>acid</sub>, ΔVHN, Change (%).
Different superscript letters indicate significant differences in columns using ANOVA and post-hoc Duncan’s multiple-comparison method.

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Differences in enamel hardness after immersion in orange juice for 2 h.
artificial saliva into the microdefect structure (pore) of the demineralized specimen surface, while the FV-indirect group formed fluorapatite using the fluoride contained in the varnish and the minerals in the artificial saliva acting together. It is considered that hydroxyapatite and fluorapatite formed in each group were responsible for the differences in acid solution resistance according to the presence or absence of FV.

Several limitations of this study should be discussed. Artificial saliva contains more minerals than actual human saliva but does not contain proteins and enzymes, which could have resulted in remineralization occurring more easily than in the real oral environment. In addition, the acid challenge test is an experimental model that reproduces the phenomenon of acid challenges mainly in vitro, and the phenomenon that occurs on the enamel surface differs from demineralization. Demineralization is caused by the surface layer being protected and the internal minerals disappearing, while the acid challenges produced the phenomenon of acid challenges mainly in vitro, and the phenomenon that occurs on the enamel surface differs from demineralization. Demineralization is considered to be due only to the action of liberated ions. Therefore, remineralization by FV is considered a short-term effect due only to the action of liberated ions. In addition, the present experiments did not reproduce salivary circulation in the oral cavity since only a simple immersion method was employed. Future studies should perform remineralization experiments after varnish application using human saliva and a pH-cycling model, or use an in situ model to demonstrate remineralization. The results of this study suggest that the FV-indirect application method results in a higher remineralization efficacy than the direct application of FV to carious lesions. It is considered that the contact with saliva and the presence of fluoride ions are involved in remineralization of initial dental caries. Further in vivo research will be required before the results obtained in this study can be applied clinically.

Conflicts of interest

The authors declare that they have no competing interests.

Acknowledgements

This work was supported by a 2-Year Research Grant of Pusan National University.

References

1. Autio-Gold JT, Courts F. Assessing the effect of fluoride varnish on early enamel carious lesions in the primary dentition. JADA 2001;132:1247–53.
2. Bawden JW. Fluoride varnish: a useful new tool for public health dentistry. J Public Health Dent 1998;58:266–9.
3. Marinho VC, Higgins J, Logan S, Sheeham A. Fluorides for preventing dental caries in children and adolescents. The Cochrane Library, 2002.
4. Bishara SE, Ostby AW. White spot lesions: formation, prevention, and treatment. Semin Orthod 2008;14:174–82.
5. Castellano J, Donly K. Potential remineralization of demineralized enamel after application of fluoride varnish. Am J Dent 2004;17:462–4.
6. Lippert F, Hara AT, Martinez-Mier EA, Zero DT. In vitro caries lesion rehardening and enamel fluoride uptake from fluoride varnishes as a function of application mode. Am J Dent 2013; 26:81–5.
7. Murakami C, Böecker M, Corrêa MSNP, Mendes FM, Rodrigues CRMd. Effect of fluoride varnish and gel on dental erosion in primary and permanent teeth. Arch Oral Biol 2009; 54:997–1001.
8. White D. Use of synthetic polymer gels for artificial carious lesion preparation. Caries Res 1987;21:228–42.
9. Wong L, Sisions C. A comparison of human dental plaque microcosm biofilms grown in an undefined medium and a chemically defined artificial saliva. Arch Oral Biol 2001;46: 477–86.
10. Attin T, Meyer K, Hellwig E, Buchalla W, Lennon A. Effect of mineral supplements to citric acid on enamel erosion. Arch Oral Biol 2003;48:753–9.
11. Seppa L, Tolonen T. Caries preventive effect of fluoride varnish applications performed two or four times a year. Euro J Oral Sci 1990;98:102–5.
12. Seppa L, Tuutti H, Luoma H. Post-treatment effect of fluoride varnishes in children with a high prevalence of dental caries in a community with fluoridated water. J Dent Res 1984;63: 1221–2.
13. Clark DC. A review on fluoride varnishes: an alternative topical fluoride treatment. Community Dent Oral Epidemiol 1982;10: 117–23.
14. Mode´er T, Twetman S, Bergstrand F. Three-year study of the effect of fluoride varnish (Duraphat) on proximal caries prevalence and treatment. J Oral Sci 1995;37:262–7.
15. Arends J, Schüthof J. Fluoride content in human enamel after fluoride application and washing—an in vitro study. Caries Res 1975;9:363–72.
16. Koulourides T, Feagin F, Pigmans W. Remineralization of dental enamel by saliva in vitro. Ann NY Acad Sci 1965;131:751–7.
17. Damato F, Strang R, Stephen K. Effect of fluoride concentration on remineralization of carious enamel in an in vitro pH-cycling study. Caries Res 1990;24:174–80.
18. Gate J, Buijs M, Damen J. pH-cycling of enamel and dentin lesions in the presence of low concentrations of fluoride. Euro J Oral Sci 1995;103:362–7.
19. Cruz R, Ógaard B, Rölla G. Uptake of KOH-soluble and KOH-insoluble fluoride in sound human enamel after topical application of a fluoride varnish (Duraphat) or a neutral 2% NaF solution in vitro. Euro J Oral Sci 1992;100:154–8.
20. Kim HH, Jeong MS, Kim SY, Kim JB, Jeong SH. Evaluation of release of fluoride from dental varnishes marketed in Korea. J Kor Acad Oral Health 2014;38:131–7 [In Korean, English abstract].
21. Vieira A, Ruben JL, Huysmans M. Effect of titanium tetrafluoride, amine fluoride and fluoride varnish on enamel erosion in vitro. Caries Res 2005;39:371–9.
22. Ten Cate J, Featherstone J. Mechanistic aspects of the interactions between fluoride and dental enamel. *Crit Rev Oral Biol Med* 1991;2:283–96.

23. Chauncey HH, Lionetti F, Winer RA, Lisanti VF. Enzymes of human saliva: I. The determination, distribution, and origin of whole saliva enzymes. *J Dent Res* 1954;33:321–34.

24. Attin T. Methods for assessment of dental erosion. In: Lussi A, ed. *Dental Erosion: from diagnosis to therapy*. Bassel: Karger Medical and Scientific Publishers, 2006:152–72.

25. Eakle WS, Featherstone JD, Weintraub JA, Shain SG, Gansky SA. Salivary fluoride levels following application of fluoride varnish or fluoride rinse. *Community Dent Oral Epidemiol* 2004;32:462–9.