

## The effect of different fluoride application methods on the remineralization of initial carious lesions

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**Objectives:** The purpose of this study was to assess the effect of single and combined applications of fluoride on the amount of fluoride release, and the remineralization and physical properties of enamel. **Materials and Methods:** Each of four fluoride varnish and gel products (Fluor Protector, FP, Ivoclar Vivadent; Tooth Mousse Plus, TM, GC; 60 Second Gel, A, Germiphene; CavityShield, CS, 3M ESPE) and two fluoride solutions (2% sodium fluoride, N; 8% tin(II) fluoride, S) were applied on bovine teeth using single and combined methods (10 per group), and then the amount of fluoride release was measured for 4 wk. The electron probe microanalysis and the Vickers microhardness measurements were conducted to assess the effect of fluoride application on the surface properties of bovine teeth. **Results:** The amount of fluoride release was higher in combined applications than in single application ( $p < 0.05$ ). Microhardness values were higher after combined applications of N with FP, TM, and CS than single application of them, and these values were also higher after combined applications of S than single application of A ( $p < 0.05$ ). Ca and P values were higher in combined applications of N with TM and CS than single application of them ( $p < 0.05$ ). They were also increased after combined applications of the S with A than after single application ( $p < 0.05$ ). **Conclusions:** Combined applications of fluoride could be used as a basis to design more effective methods of fluoride application to provide enhanced remineralization. (*Restor Dent Endod* 2016;41(2):121-129)

**Key words:** Dental Caries; Electron probe microanalysis; Fluorides; Remineralization; Vickers hardness

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### Introduction

Generally, preventive measures such as the use of fluoride to prevent dental caries are more efficient than the dental treatment. Therefore, studies have been conducted on different types of fluoride and their application methods in order to assess the effects of fluoride.<sup>1-3</sup> When fluoride products are applied to enamel surface of the teeth, the fluoride ions replace the hydroxide ions in the hydroxyapatite crystal structure of the teeth.<sup>4</sup> The lower solubility of fluorapatite compared to that of hydroxyapatite results in higher acid resistance of the enamel. Larger binding forces between the fluoride and apatite crystals increase the hardness of the teeth.<sup>5-8</sup>

When fluoride concentration in enamel is increased by topical fluoride application, this leads to the formation of a fluoride film on the enamel surface, thus reducing the penetration of acid by bacteria and enhancing remineralization.<sup>5</sup> Therefore,

topical fluoride application is used for individuals with high caries risk, orthodontic patients, and patients with decreased salivary flow.<sup>9</sup> Topical fluoride application can be classified into personal and professional applications. Sodium fluoride (NaF) solution can be applied to the teeth with a cotton applicator or by using iontophoresis. The preventive effects of 2% NaF solution against initial dental caries have been reported.<sup>1-3</sup> It has also been reported that tin(ii) fluoride (SnF<sub>2</sub>) solution has antibacterial effects,<sup>10,11</sup> but it can cause discoloration of enamel surface and gingival irritation.<sup>12</sup> In addition, fluoride gels and varnishes enhance remineralization of initial carious lesions and resist enamel demineralization. It was reported that the incidence of dental caries decreased when Fluor Protector was applied to enamel surface of bovine teeth and the teeth were exposed to cariogenic beverages.<sup>13</sup> Recently, some reports attracted attention because they showed that Tooth Mousse Plus which contains casein phosphopeptide-amorphous calcium phosphate fluoride (CPP-ACPF) increased the remineralization of initial dental caries by supplementing individual salivary buffering capacity.<sup>6,14</sup> Also, CavityShield contains a colophony component to attach on enamel surface for a longer period and maintains 5% NaF content consistently due to its viscous component. It enhanced the fluoride concentration in enamel.<sup>15</sup>

Fluoride varnish has been widely used in Canada and European countries since the 1970s. Due to the application of these products, a small amount of high density fluorides remain in contact with the teeth for a long duration, and then penetrate the tooth structure to form bonds.<sup>16</sup> However, products such as fluoride solutions, gels, or foams remain in contact with teeth for a shorter duration than varnishes, thus resulting in the formation of bonds in more superficial portion of the enamel.<sup>9,17</sup> To enhance the effects of fluoride application, contact time between teeth and fluoride should be extended, and accordingly, continuing studies on this topic have been performed widely, which has led to new findings and methods.<sup>9,16,17</sup> The uptake of fluoride by enamel is proportional to the amount of contact time.<sup>18</sup> It was reported that the application of acidulated phosphate fluoride (APF) gel on enamel surface initially resulted in high concentration of fluoride on enamel surface. Therefore, the combined application of APF-SnF<sub>2</sub> was introduced as a new method.<sup>17,19</sup>

In this study, single or combined applications of fluoride were suggested to protect against dental caries by increasing the effects of fluoride application. The null hypothesis of this study was that single and combined applications of different topical fluoride products exert the same remineralization effects on enamel.

## Materials and Methods

### Preparation of specimens and formation of artificial caries

Thirty fresh bovine mandibles were obtained from a local slaughterhouse (Chuklim, Iksan, Korea) and were cryopreserved in order to maintain the physical properties of the bovine teeth. Eight incisors were extracted from each mandible. From these, flat, smooth, sound (non-carious) teeth were collected for use in this study. The bovine teeth were cut into block-shaped specimens of 8 × 8 mm<sup>2</sup> size ( $n = 240$ ) to obtain the widest and flattest areas on the labial surfaces. The specimens were polished to a gloss using # 400 - 1,200 grit silicon carbide papers (Deerfos, Incheon, Korea) and 0.5 μm alumina oxide suspension (Buehler, Lake Bluff, IL, USA). Polished samples were immersed in a demineralization solution (pH 5.0) containing 0.2% carbopol (BF Goodrich, Cleveland, OH, USA) and 0.1% lactic acid (Sigma-Aldrich Chemical, St. Louis, MO, USA) saturated with calcium phosphate tribasic for 72 hours. Demineralized specimens with a Vickers hardness number (VHN) of  $96 \pm 0.4$  were selected for standardization ( $n = 130$ ).

### Measurement of Vickers microhardness

Vickers microhardness of the demineralized specimens and the fluoride treated specimens was measured using a digital microhardness tester (HM-124, Mitutoyo, Kanagawa, Japan) with a load of 200 g for 10 seconds in four areas (4 per group).

### Topical fluoride application

In the control group, no treatments were done on enamel surfaces of specimens, and in the experimental groups, four products including Fluor Protector (FP, Ivoclar Vivadent, Schaan, Liechtenstein), Tooth Mousse Plus (TM, GC, Tokyo, Japan), 60 Second Gel (A, Germiphene, Brantford, Canada), CavityShield (CS, 3M ESPE, St. Paul, MN, USA) were used for single application of fluoride (Table 1). For combined applications of fluoride, 2% sodium fluoride (N, Junsei, Tokyo, Japan), 8% tin(ii) fluoride (S, Sigma-Aldrich Chemical, St. Louis, MO, USA) solutions were prepared, and after application of the 4 fluoride products, the specimens were immersed in each solution for 1 minute (Table 2). In case of single application of fluoride, the specimens were washed with distilled water and dried. The remaining surfaces of the specimens, other than enamel surfaces on which fluoride was applied, were covered with nail varnish. The prepared specimens were placed in a tightly capped container filled with 5 mL of distilled water and stored in an incubator at 37°C.

**Table 1.** Fluoride products used in this study

Product	Code	Manufacturer	Ingredient
Fluor Protector	FP	Ivoclar Vivadent, Schaan, Liechtenstein	(Bis{4-[2-(difluorhydroxysilyl)ethyl]-2-methoxycyclohexyl}[N,N-(trimethylhexane-1,6-diyl)dicarbamate])
Tooth Mousse Plus	TM	GC, Tokyo, Japan	Casein Phosphopeptide-Amorphous Calcium Phosphate Fluoride
60 Second Gel	A	Germiphene, Brantford, Canada	1.23% Acidulated phosphate fluoride
CavityShield	CS	3M ESPE, St. Paul, MN, USA	5% Sodium fluoride
Sodium Fluoride	N	JUNSEI, Tokyo, Japan	Sodium fluoride
Tin(II) Fluoride	S	Sigma-Aldrich Chemical, St. Louis, MN, USA	Stannous fluoride

**Table 2.** Fluoride application methods on specimens followed in this study ( $n = 10$ )

Group	Fluoride application method (application time)
Non	Untreated
FP	Fluor Protector (1 min)
FPN	Fluor Protector (1 min) + Immersion in 2% Sodium Fluoride (1 min)
FPS	Fluor Protector (1 min) + Immersion in 8% Tin(II) Fluoride (1 min)
TM	Tooth Mousse Plus (3 min)
TMN	Tooth Mousse Plus (3 min) + Immersion in 2% Sodium Fluoride (1 min)
TMS	Tooth Mousse Plus (3 min) + Immersion in 8% Tin(II) Fluoride (1 min)
A	60 Second Gel (1 min)
AN	60 Second Gel (1 min) + Immersion in 2% Sodium Fluoride (1 min)
AS	60 Second Gel (1 min) + Immersion in 8% Tin(II) Fluoride (1 min)
CS	CavityShield (4 hr)
CSN	CavityShield (4 hr) + Immersion in 2% Sodium Fluoride (1 min)
CSS	CavityShield (4 hr) + Immersion in 8% Tin(II) Fluoride (1 min)

### Measurement of fluoride release

The amount of fluoride release was measured on days 1, 2, 3, 4, 7, 14, 21, and 28 (5 per group). Distilled water was changed everyday throughout the experiment. Five milliliters of the solution that contained the specimen was mixed with the same amount (5 mL) of TISAB III (Total Ionic Strength Adjustment Buffer, Thermo Scientific Orion, Beverly, MA, USA). This solution (total volume = 10 mL) was measured on a fluoride electrode (Orion 96-09BN, Thermo Scientific Orion) with a millivolt meter (Orion720A plus, Thermo Electron, San Jose, CA, USA). TISAB III solution was added to maintain its pH in the range of 5.0 - 5.5 because the fluoride electrode is sensitive to changes in pH. To measure the amount of

fluoride released, 0.1, 1, 10, and 100 ppm of standard fluoride solutions (Fluoride standard, Thermo Scientific Orion) were used to standardize the measurements.

### Electron probe micro analysis

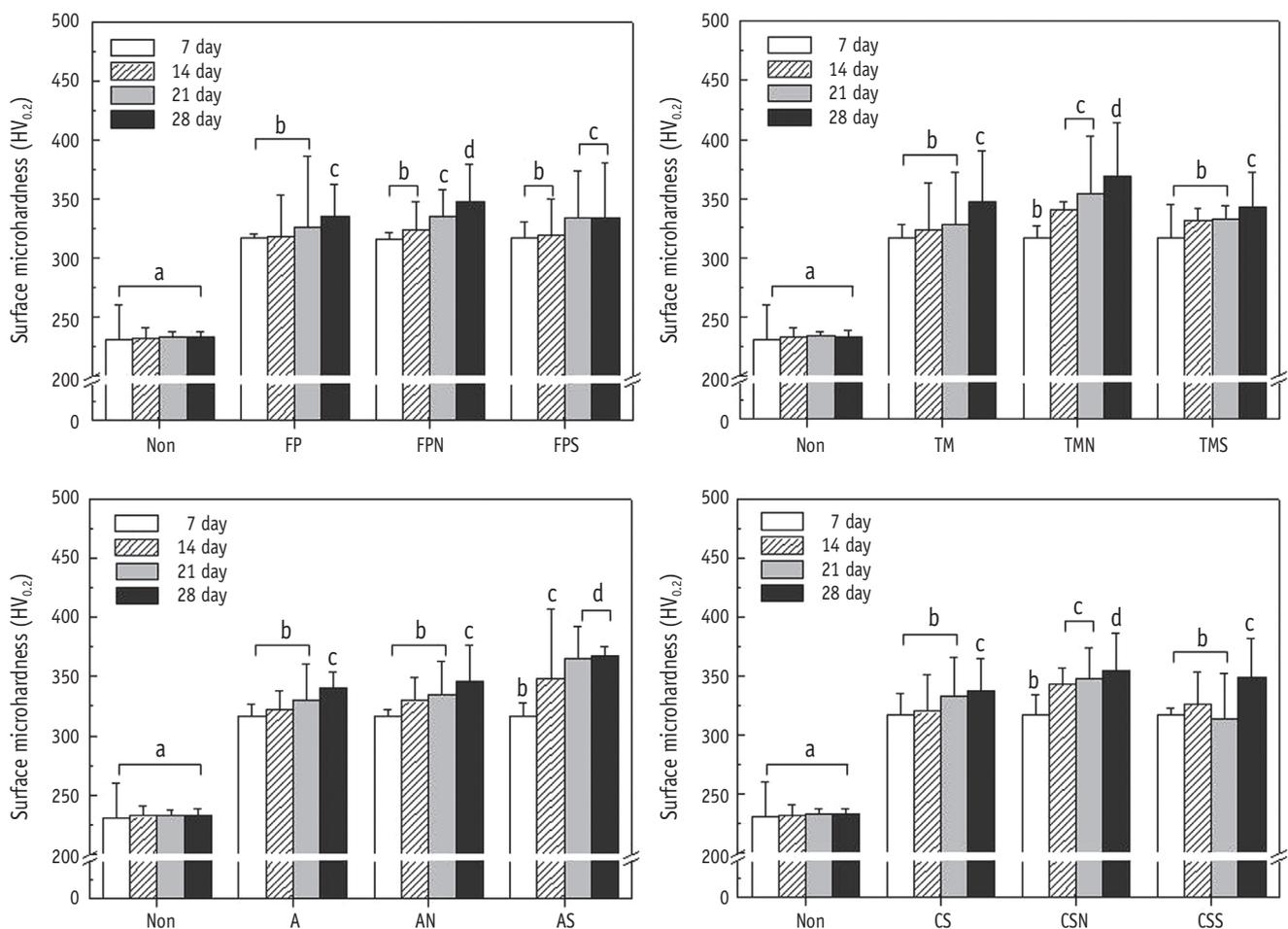
Changes in the constituents of enamel surface caused by fluoride uptake due to fluoride treatment were analyzed on days 7, 14, 21, and 28 by conducting quantitative analysis of principal components of teeth (Ca and P) using electron probe microanalysis (EPMA, EPMA-1600, Shimadzu, Kyoto, Japan) (1 per group). In the EPMA, 10  $\mu\text{m}$  beam size, 15 kV acceleration voltage, and 20 nA electron beam current were applied, and the quantitative analysis was conducted at four points.

### Statistical analysis

All the data was analyzed using SPSS 12.0 (SPSS, Chicago, IL, USA). To compare the remineralization effects of single and combined applications of fluoride, two-way repeated measure ANOVA tests were conducted, and variables showing significant differences were tested subsequently by Tukey's combined comparison tests. A  $p$  value of 0.05 was considered statistically significant.

### Results

Measurements of Vickers microhardness of the specimen surfaces are presented in Figure 1. Among the groups in which FP was applied, the FPN group showed the highest values in surface microhardness after 4 weeks ( $p < 0.05$ ). The TMN and CSN groups also showed significantly higher values after 4 weeks than TMS and CSS groups ( $p < 0.05$ ). However, among the groups in which A was applied, the AS group showed a significant increase in surface microhardness after 3 and 4 weeks compared to the AN group ( $p < 0.05$ ).



**Figure 1.** Mean Vickers microhardness of specimen surface after fluoride applications ( $n = 4$ ). Bars indicate standard deviations. Non, Untreated; FP, Fluor Protector; FPN, Fluor Protector + 2% sodium fluoride (NaF); FPS, Fluor Protector + 8% tin(ii) fluoride (SnF<sub>2</sub>); TM, Tooth Mousse Plus; TMN, Tooth Mousse Plus + 2% NaF; TMS, Tooth Mousse Plus + 8% SnF<sub>2</sub>; A, 60 Second Gel; AN, 60 Second Gel + 2% NaF; AS, 60 Second Gel + 8% SnF<sub>2</sub>; CS, CavityShield; CSN, CavityShield + 2% NaF; CSS, CavityShield + 8% SnF<sub>2</sub>.

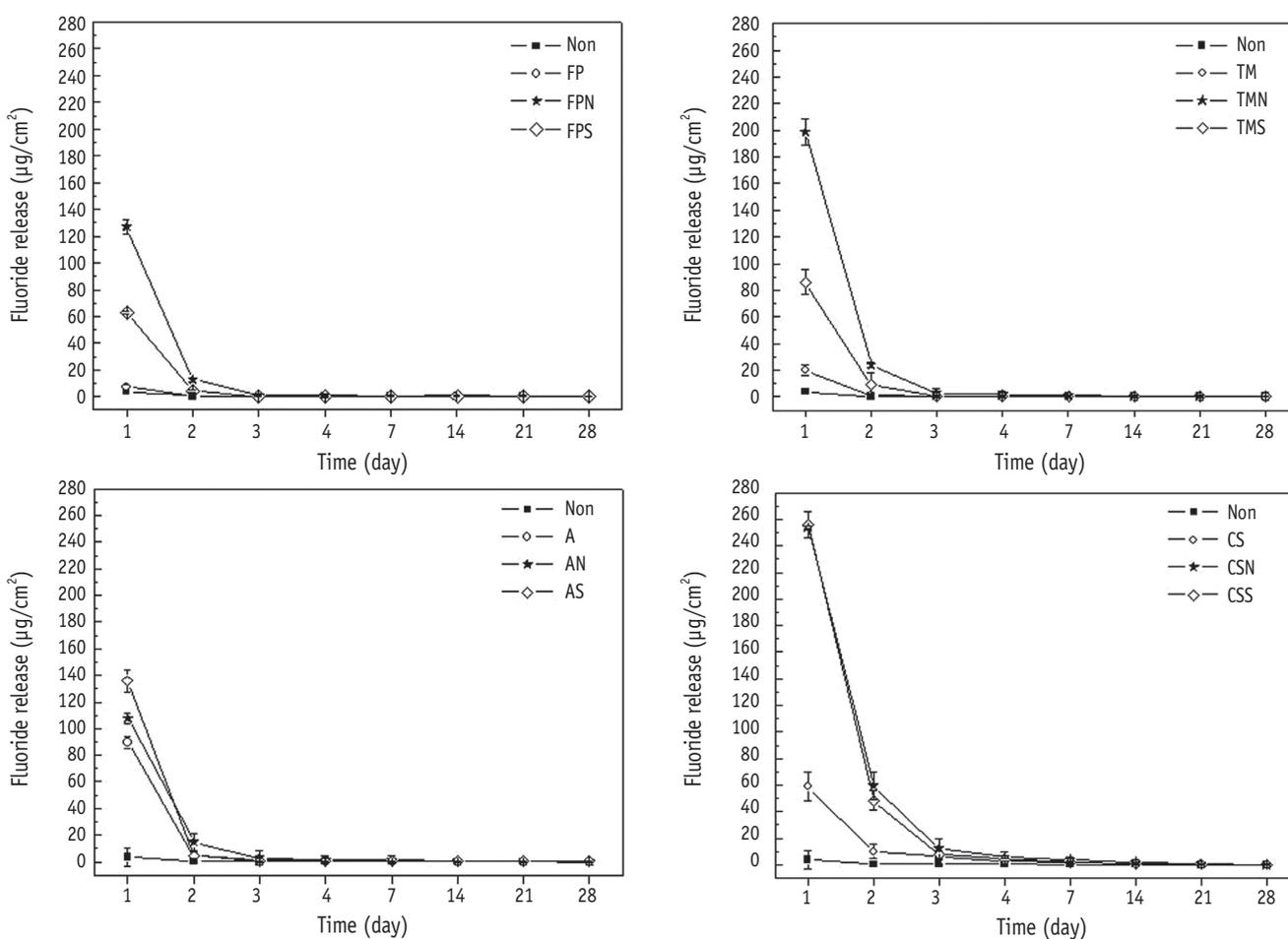
a - d, Groups with different letters were significantly different ( $p < 0.05$ ).

HV<sub>0.2</sub>, Hardness values were determined using a digital microhardness tester with a load of 0.2 kg.

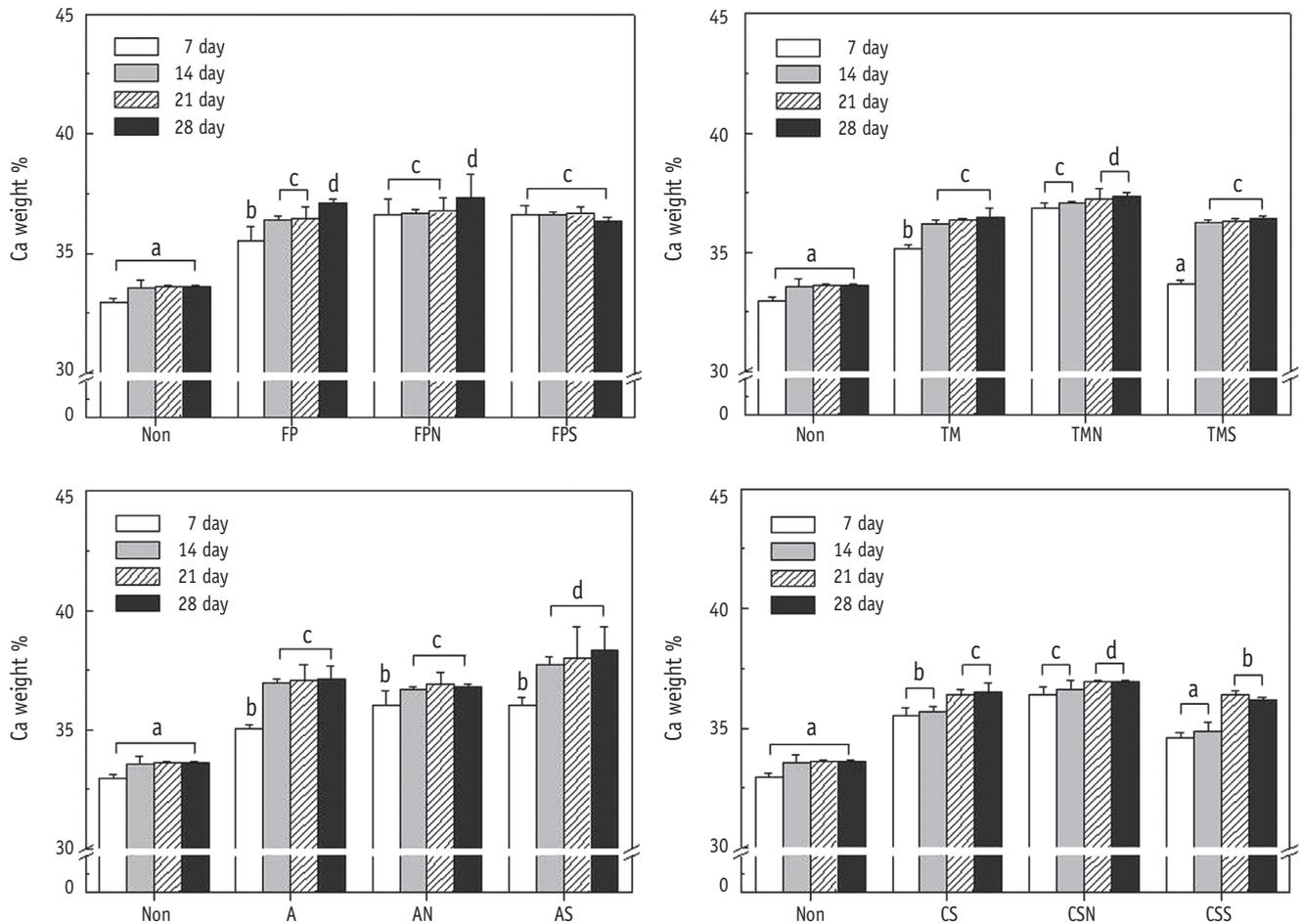
Changes in fluoride release at each time point are illustrated in Figure 2. Compared with the non-treatment group, fluoride release was significantly higher on the first day in all of the treated groups ( $p < 0.05$ ), but decreased rapidly after the first day, and then continued to decrease rather slowly. It is clear that among the groups in which FP, TM, and CS were applied, that fluoride release was significantly higher in the groups treated with combined application of N and S. In particular, a large fluoride

release was observed in the FPN, TMN, and CSN groups. However, among the groups in which A was applied, the fluoride release was significantly higher in the AS group than AN group ( $p < 0.05$ ).

Changes in the principal components of the teeth were observed by measurements of Ca and P after fluoride application (Figures 3 and 4). The measured values of Ca and P were greater in the FPN, TMN, AS, and CSN groups than those in the FPS, TMS, AN, and CSS groups ( $p < 0.05$ ).



**Figure 2.** Fluoride release after fluoride applications ( $n = 5$ ). Bars indicate standard deviations. Non, Untreated; FP, Fluor Protector; FPN, Fluor Protector + 2% sodium fluoride (NaF); FPS, Fluor Protector + 8% tin(ii) fluoride ( $\text{SnF}_2$ ); TM, Tooth Mousse Plus; TMN, Tooth Mousse Plus + 2% NaF; TMS, Tooth Mousse Plus + 8%  $\text{SnF}_2$ ; A, 60 Second Gel; AN, 60 Second Gel + 2% NaF; AS, 60 Second Gel + 8%  $\text{SnF}_2$ ; CS, CavityShield; CSN, CavityShield + 2% NaF; CSS, CavityShield + 8%  $\text{SnF}_2$ .



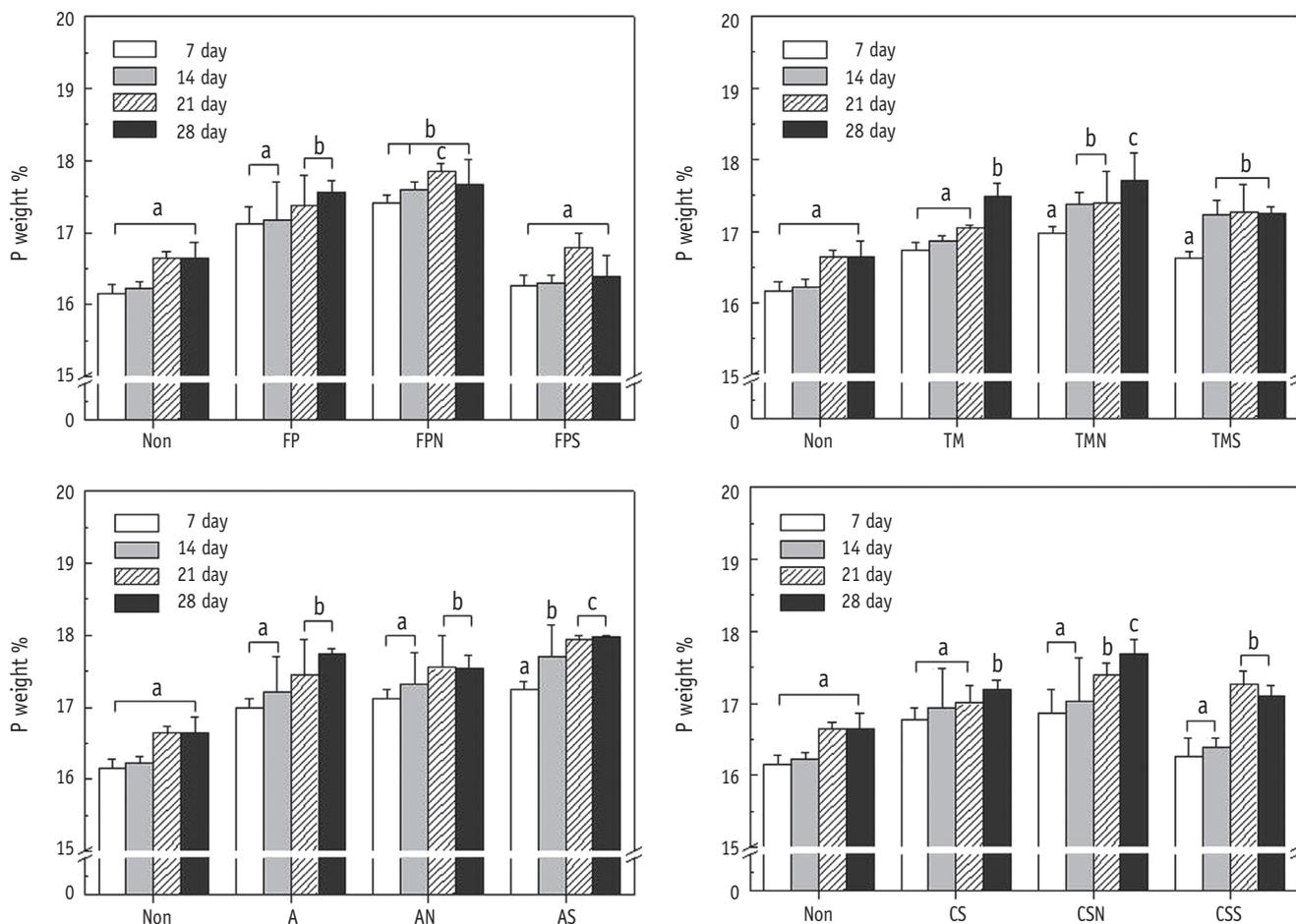
**Figure 3.** Calcium content of specimens after fluoride applications by EPMA analysis. Bars indicate standard deviations. Non, Untreated; FP, Fluor Protector; FPN, Fluor Protector + 2% sodium fluoride (NaF); FPS, Fluor Protector + 8% tin(ii) fluoride (SnF<sub>2</sub>); TM, Tooth Mousse Plus; TMN, Tooth Mousse Plus + 2% NaF; TMS, Tooth Mousse Plus + 8% SnF<sub>2</sub>; A, 60 Second Gel; AN, 60 Second Gel + 2% NaF; AS, 60 Second Gel + 8% SnF<sub>2</sub>; CS, CavityShield; CSN, CavityShield + 2% NaF; CSS, CavityShield + 8% SnF<sub>2</sub>.

a - d, Groups with different letters were significantly different ( $p < 0.05$ ).

## Discussion

To identify the effects of topical fluoride application and combined applications with fluoride solution on teeth, studies assessing amount of fluoride released, changes in surface microhardness, and changes in the Ca and P components of tooth enamel using the EPMA have been conducted.<sup>6,7,18,20-24</sup> Crall *et al.* reported that preventive effects against dental caries increased along with an increase in the amount of fluoride released after combined application of SnF<sub>2</sub> following the application of APF.<sup>19</sup> In addition, Reynolds *et al.* reported that the

remineralization rate was higher after the combined application of NaF solution than the in situ single application of CPP-ACP fluoride products, and it was accompanied with a decrease in the rate of dental caries.<sup>25</sup> In this study, fluoride release was measured continuously from the first day after fluoride application. Specifically, a large amount of fluoride was released in the FPN, TMN, and CSN groups treated with combined application of N compared to the FP, TM, and CS groups ( $p < 0.05$ ). Also, the amount of fluoride was significantly higher in the AS group after combined application of S, compared to the A group ( $p < 0.05$ ). Fluoride release



**Figure 4.** Phosphorus content of specimens after fluoride applications by EPMA analysis. Bars indicate standard deviations. Non, Untreated; FP, Fluor Protector; FPN, Fluor Protector + 2% sodium fluoride (NaF); FPS, Fluor Protector + 8% tin(ii) fluoride (SnF<sub>2</sub>); TM, Tooth Mousse Plus; TMN, Tooth Mousse Plus + 2% NaF; TMS, Tooth Mousse Plus + 8% SnF<sub>2</sub>; A, 60 Second Gel; AN, 60 Second Gel + 2% NaF; AS, 60 Second Gel + 8% SnF<sub>2</sub>; CS, CavityShield; CSN, CavityShield + 2% NaF; CSS, CavityShield + 8% SnF<sub>2</sub>. a - c, Groups with different letters were significantly different ( $p < 0.05$ ).

at each time point after fluoride application showed a rapid decrease from the first day to the next day. The initial burst of fluoride ion release after topical fluoride application has been explained by diffusion from enamel surface of high fluoride content. As assumed in the studies by Lee *et al.*,<sup>7</sup> the amount of fluoride release would depend on the differences in solubility and release of fluoride according to the fluoride products. An increase in fluoride release on enamel was observed in cases with combined applications of fluoride such as A with S, and N with FP, TM, and CS compared to cases with a single application of fluoride.

Densified enamel due to increased fluoride content through fluoride application increases the strength of the tooth structure, and such increases have been observed in the changes in microhardness of enamel surface for 4 weeks after topical fluoride application in many studies.<sup>6,7,17,18,20</sup> Kodaka *et al.* reported that there existed a close relationship between mineral contents and the microhardness of enamel of sound tooth,<sup>22</sup> and Feagin *et al.* noted that the increase in the Ca and P concentrations increase the strength of tooth structure with an accelerated remineralization rate.<sup>24</sup> Among various mineral contents that constitute tooth enamel, Ca and P form the

apatite through a deposition process called calcification or mineralization.<sup>26</sup> As the remineralization facilitated through fluoride application is expected to cause changes in the Ca and P contents and the EPMA is known to be capable of measuring changes in these constituents rather accurately.<sup>21,27</sup> In this study, tooth hardness observed through changes in the microhardness after single and combined applications of fluoride was increased in the groups treated with combined applications of fluoride with N (FPN, TMN, and CSN groups) compared to the groups treated with single applications of FP, TM, and CS. Also, the group treated with combined application of fluoride with S (AS group) showed significantly increased microhardness compared to the A group ( $p < 0.05$ ). This may suggest that tooth hardness was greatly enhanced in cases in which combined applications of fluoride using A with S and FP, TM, and CS with N. Also, the EPMA results showed the significant increase in Ca and P contents, which allowed significant enhancement of tooth hardness in the cases with combined applications (FPN, TMN, CSN, and AS groups) compared to single applications (FP, TM, CS, and A groups) ( $p < 0.05$ ).

To summarize the results, the time required for binding fluorides to tooth structure was increased by combined applications of fluoride using S with A and N with FP, TM, and CS. This resulted in an increase in fluoride contents on the surface and inside the teeth. Also, the increase in the fluoride content of enamel surface improved the hardness of the tooth structure. These results corresponded partially with the results reported by Crall *et al.*, which demonstrated that the combined application of A and S resulted in excellent preventive effects against initial dental caries compared to cases treated with a single application of fluoride with APF.<sup>28</sup>

Since most of the current professional fluoride applications include single fluoride products, we aimed to achieve an increased uptake of fluoride by applying fluorides for a longer time through combined applications of fluoride. The combined applications of fluoride adopted in this study could be used as a basis for designing new methods of fluoride application for prevention against dental caries. However, there could be differences between the real oral environment and experimental conditions, and there were also time limitation of 4 weeks for each measurements. Therefore, more comprehensive and in-depth studies on the long-term remineralization effects in situ or clinical tests are needed.

## Conclusions

Combined topical applications of fluoride used in this study showed improved remineralization effects and strengthening enamel. It was also assumed that the combined applications of fluoride products and solutions

could be used as the basis for designing more effective methods of fluoride application to protect against dental caries.

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Conflict of Interest: No potential conflict of interest relevant to this article was reported.

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