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Analysis of Tooth Surface Changes after Treatment with Silver Fluoride Using Energy-Dispersive Electromagnetic Radiation Spectroscopy

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This study sought to examine tooth surface changes and remineralization after treatment with 38 % water-based silver fluoride (AgF) solution and potassium iodide (KI), which are known to have teeth-remineralization effects. Tooth samples were prepared by exposing enamel and dentin. Riva Star Aqua (SDI, Bayswater, Australia), consist of AgF and KI ,was applied to tooth surfaces, after treatment, stored in artificial saliva for 7 or 14 days. Using scanning electron microscopy, changes in tooth surface shape and remineralization were assessed. Our analysis revealed that upon treatment with AgF for 7 or 14 days, the dentin surface appeared very smooth, indicating remineralization. Analysis of the levels of calcium (Ca), phosphorous (P), and argentum (Ag) using electromagnetic radiation spectroscopy revealed changes in the levels of Ca and P in the enamel and that after 14 days, there were significant changes in the levels of Ag in dentin. These findings indicate that the agent applied using AgF and KI led to teeth remineralization, highlighting them as effective remineralization materials.

Keywords : energy-dispersive electromagnetic radiation spectroscopy, scanning electron microscope, silver diamine fluoride, tooth surface change, magnetic component analysis

1. Introduction

Dental caries occurs when oral microorganisms ferment carbohydrates into lactic acid, which dissolves enamel through the process of demineralization, and it is mainly prevented using fluoride. In enamel, fluoride forms fluorapatite, which increases the acid resistance of enamel and reduces dental caries [1, 2]. Detecting caries lesions early and controlling the balance between tooth demineralization and remineralization is crucial in preventing and managing dental caries [1]. To enhance tooth remineralization, fluoride is included in various products for preventing dental caries, including fluoride varnish, which is widely used because of its high adhesion and low risk of being swallowed [3]. Although silver fluoride (AgF) is a widely used fluoride agent, its applications are limited by its instability and the risk of fluorosis because of high fluoride concentrations [4]. However, despite its disadvantages, such as tooth discoloration and a metallic taste, AgF can block the formation and progression of dental caries. Therefore, in this study, we used an US Food and Drug Administration -approved, water-based AgF product with improved stability because of added ammonia [5]. AgF is composed of a diamine silver ion and a fluorine ion, which are ammonia molecules combined with silver ions. The silver ions deposit silver salts on tooth surfaces, where they exert antimicrobial effects by directly damaging bacterial cell membranes, denaturing proteins, and inhibiting bacterial gene transcription [6]. AgF effectively inhibits caries progression in infants and children. Typically, to effectively stop caries in infants and children, AgF is applied once a year and is more effective than applying fluoride varnish at 3-month intervals. This regimen has been reported to effectively cause tooth remineralization and prevent caries progression [7]. A study comparing AgF, sodium fluoride (NaF) varnish, and Acidulated Phosphate Fluoride found AgF to be the most effective at inhibiting *Streptococcus*

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mutans which is main bacteria occuring dental caries [8]. However, because it causes tooth discoloration, water-based AgF is applied along with potassium iodide (KI). The reaction between AgF and hydroxyapatite, a tooth component, produces silver phosphate and calcium fluoride, with the residual silver ions present in the silver phosphate causing discoloration and turning black. It has been shown that upon application, KI reacts with the remaining silver phosphate, thereby reducing discoloration [9]. Taking advantage of the benefits of ammonia-based Silver Diamine Fluoride, water-based AgF (Riva Star Aqua™, SDI) was recently developed. In addition to its antibacterial properties, AgF is also effective at suppressing and removing biofilms [10]. Here, after applying the water-based AgF solution, KI was applied to tooth surfaces followed by the analysis of remineralization and tooth surface changes after 7 and 14 days, respectively.

2. Materials and Methods

2.1. Sample preparation

Extracted human premolars were inspected under a stereomicroscope (SZ-CTV, Olympus, Tokyo, Japan) and teeth with morphological abnormalities were excluded from further analysis. A total of 15 tooth crowns were sagittally cut using a hard-tissue cutter (Struers Minitom, Denmark) and embedded in 5.1 × 5.1 × 3.0 cm paraffin blocks. The surface was then polished sequentially using 400-1800 grit abrasive paper to obtain a parallel to tooth long axis and uniform surface (Fig. 1).

2.2. Drug treatment

Riva Star Aqua (SDI, Bayswater, Australia), which is approved by the Korean Ministry of Food and Drug Safety, was used in two steps: step 1 (38 % water-based AgF solution) and step 2 (KI). It was then used to prevent tooth hypersensitivity without discoloration. The tooth samples were then divided into the control group, which was incubated in artificial saliva, and the experimental group, to which 17 µl of Riva Star Aqua (step 1) were applied using a sterile micro brush for 2 min, followed by the application of 20 µl of step 2 Riva Star Aqua according to manufacturer instructions. Next, the experimental samples were washed thrice using sterile distilled water and incubated in artificial saliva for 7 or 14 days, repectively.

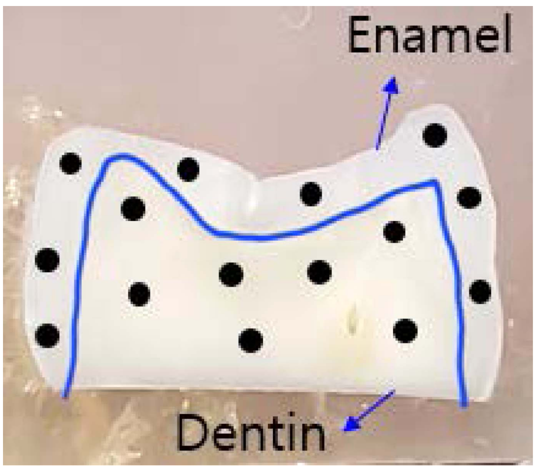

Fig. 1. (Color online) Preparation of test specimens and detection points for dentin and enamel.

2.3. Scanning electron microscopy

To assess the formation of hydroxyapatite and microscopic changes on the enamel and dentin surfaces, the samples' surfaces were dried, coated with platinum, and mounted. The surfaces of the samples from each group were then examined using scanning electron microscopy (SEM, JSM-IT500, JEOL, Tokyo, Japan) at a magnification of 1,000x, at 15 kV.

2.4. Energy-dispersive electromagnetic radiation spectroscopy

Energy-dispersive electromagnetic radiation spectroscopy (EDS; JSM-IT500, JEOL, Tokyo, Japan) was used to analyze the main enamel and dentin surface components were analyzed using. The samples' surfaces were first completely dried at room temperature, coated with platinum, and then fixed on a stand using adhesive tape. Surface spot sizes were detected at 400 µm, at an acceleration voltage of 0.1 eV and 15 kV. The levels of Ca, P, and Ag were expressed as average weight % per surface of enamel or dentin. The energy of the X-ray photon is associated with the radiation frequency as described in the equation below.

$$E = h\nu$$

h = Planck's constant (6.626×10^{-34} J/s or 4.135×10^{-15} eV/s)
ν = frequency

The wavelength λ is related to the photon energy as described in the equation below.

$$\lambda = 1.240 \times 10^{-6} / E$$

2.5. Statistical analysis

Statistical analysis were done using SPSS version 20.0 (SPSS, Chicago, IL, USA). Differences in the levels of Ca, P, and Ag in the experimental groups were compared

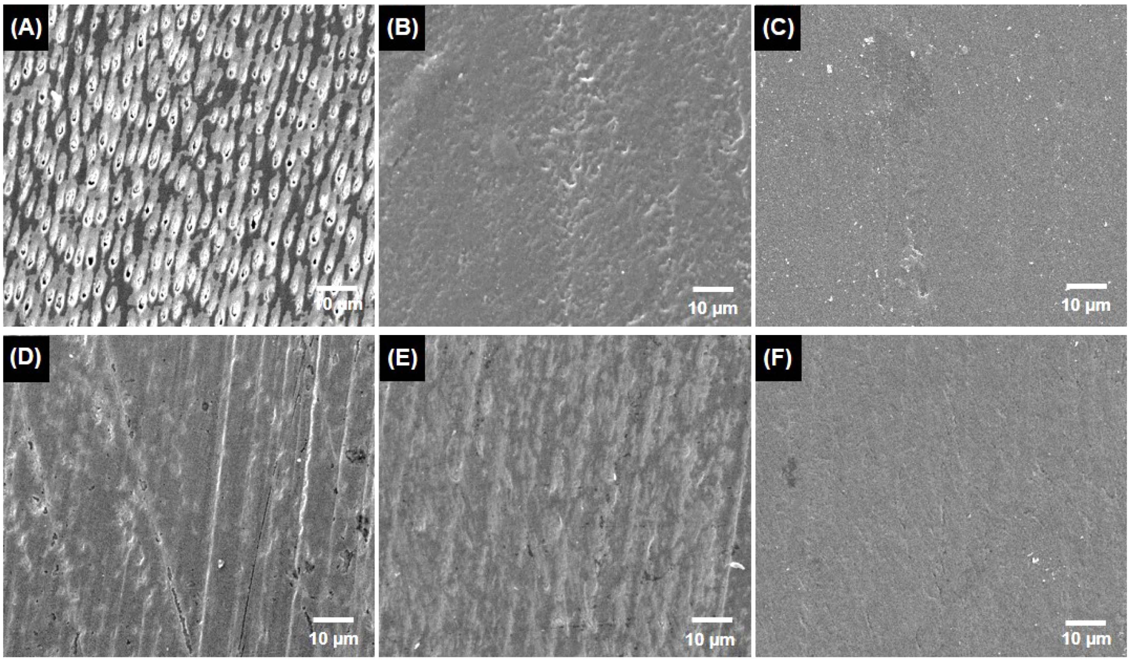

Fig. 2. (Color online) The Observation of surface changes in tooth enamel and dentin according to AgF treatment by using SEM. (A) Dentin control (B) Dentin AgF-treat 7 d (C) Dentin AgF-treat 14 d (D) Enamel control (E) Enamel AgF-treated 7 d (F) Enamel AgF-treated 14 d.

using one-way analysis of variance (ANOVA) complemented by Tukey's test ($P < 0.05$).

3. Results

3.1. Morphological analysis of dental samples' surfaces

SEM examination of the enamel and dentin surfaces after treatment with AgF for 7 or 14 days revealed that the dentinal tubules on dentin surfaces were completely closed after seven days and that a smooth dentin surface had formed after 14 days. Additionally, the cracked enamel surfaces appeared smooth after 14 days of AgF treatment (Fig. 2).

3.2. Quantification of mineral atomic components

Next, EDS was attached to the electron microscope series and then used to assess changes in the levels of Ca, P, and Ag. This analysis revealed that the levels of Ca and P, the main mineral components of enamel tissue, as well as the proportion of the mineral component of the enamel, decreased in a time-dependent manner in the dentin groups. This analysis revealed that the levels of Ag, the main component of AgF, were significantly increased in dentin after 14 days of AgF treatment. However, there was no significant difference in the enamel group ($P < 0.05$).

4. Discussion

Silver diamine fluoride (SDF) has been used to prevent dental caries for many years. SDF is used at a concentration of 38% and contains fluoride at 44,880 ppm [11]. By inhibiting glycosyltransferase activity, silver ions exert antibacterial effects by impairing bacterial metabolism, thereby inhibiting bacterial biofilm formation [6].

Fluoride, which prevents dental caries, can change hydroxyapatite to fluorapatite via ion exchange [12]. Although SDF use is limited because of its side effects, including tooth discoloration, its ability to prevent dental caries is thought to be almost the same as that of NaF varnish [13]. To minimize side effects, treatment with SDF is followed by KI application. When compared with conventional RIVA STAR, which contains SDF in an ammonia base, the RIVA STAR aqua product contains AgF in a water base and has a lower, physiological pH. In this study, we used RIVA STAR aqua, which contains both AgF and KI.

Clinically, AgF is used to desensitize hypersensitive teeth, to prevent dental caries, and to promote tooth remineralization. Here, based on manufacturer instructions, one drop (17 μl) of AgF solution was applied to each tooth for two minutes after completely drying the tooth surfaces. A previous study found that 1 μl of SDF is sufficient for a surface area of 3 mm^2 [11]. Therefore, in

Table 1. The changes in composition of dentin mineral element according to AgF treatment.

Element	Experimental group	N	Mean (at. %)	SD	p-value
P	Control	15	38.59[a]	0.95	0.040
	7 days	15	39.87[a]	1.00	
	14 days	15	35.07[b]	2.67	
Ca	Control	15	61.30[a]	0.93	0.040
	7 days	15	59.58[a]	0.95	
	14 days	15	56.17[b]	1.57	
Ag	Control	15	0.11[a]	0.15	0.001
	7 days	15	0.55[a]	0.26	
	14 days	15	8.76[b]	3.65	

[a,b)]Difference letter indicates statistics significant difference by One-Way ANOVA ($p < 0.05$).

Table 2. The changes in composition of enamel mineral element according to AgF treatment.

Element	Experimental group	N	Mean (at. %)	SD	p-value
P	Control	15	38.51	1.02	0.204
	7 days	15	38.76	0.44	
	14 days	15	38.28	0.89	
Ca	Control	15	61.37	0.90	0.625
	7 days	15	60.88	2.19	
	14 days	15	61.54	0.86	
Ag	Control	15	0.12	0.17	0.348
	7 days	15	0.65	1.62	
	14 days	15	0.18	0.20	

this study, we applied 17 μl of AgF to cover a sample surface area of 51 mm^2. Immediately after AgF application, two drops (20 μl) of KI solution were applied on the tooth surfaces as per the manufacturer's instructions, until the creamy appearance cleared.

Our SEM analysis revealed that the AgF-induced hard tissue remineralization was more significant in the dentin group. Although AgF improved surface roughness in the enamel group, the effect was not significantly different when compared with the dentin group. Although several studies indicate that SDF triggers dentin remineralization, this study found that enamel surface changes also occurred after AgF treatment [14-16].

Analysis of the mineral atomic components using EDS showed that the proportions of Ca, P, and Ag atoms were changed in the dentin and enamel groups. Targeting electrons from an electron microscope on a material causes it to release ions from its surface. Among these ions, EDS detects only the characteristic released X-rays and then displays them on the screen for energy band of the beam, respectively [17, 18].

Our EDS analysis revealed changes in the levels of Ca and P, the main mineral components of enamel and dentin, as well as in the level of Ag, the main component of AgF. As shown in Table 1, after treatment for 14 days, the levels of Ca and P atoms were significantly reduced in dentin, whereas the proportion of Ag was markedly elevated. Table 2 shows that the differences in Ca, P, and Ag atoms were not significant. Previous findings indicate that the levels of Ag upon SDF–GSH (glutathione) co-treatment were higher than after SDF treatment [19]. There's a necessity to conduct subsequent research reflected these results in previous study. This observation warrants further analysis.

To our knowledge, this is the first study to assess the levels of the atomic components of dentin and enamel after AgF application. Although AgF is a good anti-dental caries agent, few previous studies have observed its association with enamel and dentin remineralization, as well as changes in their atomic composition. Therefore, this study reports initial findings on the effects of AgF on different types of tooth hard tissues.

5. Conclusion

The application of AgF and KI effectively improved enamel and dentin remineralization in a time-dependent manner. The effect of remineralization by AgF was higher dentin than enamel. The main atomic component of AgF, Ag, markedly accumulated in dentin but it did not change significantly in enamel. Also, There was no significant changes in components of tooth hard tissue such as P and Ca elements.

References

[1] R. H. Selwitz, A. I. Ismail, and N. B. Pitts, Lancet. **369**, 51 (2007).

[2] C. M. Carey, J. Evid. Based. Dent. Pract. **14**, 13 (2014).

[3] T. Rashed, N. Alkhalefa, A. Adam, and A. AlKheraif, Int. J. Clin. Pract. **2022**, 8635254 (2022).

[4] C. Pushpalatha, K. V. Bharkhavy, A. Shakir, D. Augustine, S. V. Sowmya, H. A. Bahammam, S. A. Bahammam, N. H. Mohammad Albar, B. Zidane, and S. Patil, Front Bioeng Biotechnol. **10**, 931327 (2022).

[5] J. O. Burgess and P. M. Vaghela, Adv. Dent. Res. **29**, 131 (2018).

[6] F. M. Zheng, I. G. Yan, D. Duangthip, S. S. Gao, E. C. M. Lo, and C. H. Chu, Jpn. Dent. Sci. Rev. **58**, 249 (2022).

[7] V. Contreras, M. J. Toro, A. R. Elias-Boneta, and A. Encarnacion-Burgos, Gen. Dent. **65**, 22 (2017).

[8] V. B. Shalin Shah, Karthik Venkataraghavan, Prashant Choudhary, M Ganesh, Krishna Trivedi, Indian J. Dent. Res. **24**, 7 (2013).

[9] K. E. Lee, M. Erdenebulgan, C. M. Kang, H. I. Jung, and J. S. Song, Materials (Basel). **15**, (2022).

[10] F. Ahrari, N. Eslami, O. Rajabi, K. Ghazvini, and S. Barati, Dent. Res. J. **12**, 1 (2015)

[11] H. W. Park, H. N. Kim, J. H. Lee, and S. Y. Lee, J. Korean Acad. Pediatr. Dent. **49**, 9 (2022).

[12] M. L. Mei, E. C. M. Lo, and C. H. Chu, J. Dent. Res. **97**, 751 (2018).

[13] R. J. Wierichs, S. Stausberg, J. Lausch, H. Meyer-Lueckel, and M. Esteves-Oliveira, Caries Res. **52**, 199 (2018).

[14] M. Sayed, Y. Tsuda, K. Matin, A. Abdou, K. Martin, M. F. Burrow, and J. Tagami, Sci. Rep. **10**, 19884 (2020).

[15] M. S. Cury, C. B. Silva, R. D. Nogueira, M. G. D. Campos, R. G. Palma-Dibb, and V. R. Geraldo-Martins, Lasers. Med. Sci. **33**, 257 (2018).

[16] C. Cifuentes-Jimenez, P. Alvarez-Lloret, C. Benavides-Reyes, S. Gonzalez-Lopez, A. B. Rodriguez-Navarro, and M. V. Bolanos-Carmona, J. Adhes. Dent. **23**, 557 (2021).

[17] M. Shimizu, N. Matsui, M. Sayed, H. Hamba, S. Obayashi, M. Takahashi, Y. Tsuda, T. Takagaki, T. Nikaido, and J. Tagami, Dent. Mater. J. **40**, 1041 (2021).

[18] M. H. SeoulHee Nam, J. Magn. **25**, 6 (2020).

[19] M. Sayed, N. Matsui, N. Hiraishi, T. Nikaido, M. F. Burrow, and J. Tagami, Int. J. Mol. Sci. **19**, (2018).