Retention Improvement in Fluoride Application with Cold Atmospheric Plasma

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Abstract

This study aimed to apply fluoride formulations to enamel with cold atmospheric plasma (CAP) and analyze the fluoride uptake, retention, and acid resistance quantitatively. Human enamel specimens were divided randomly into 2 groups: group APF1, 1.23% acidulated phosphate fluoride (APF) gel; group APF2, 1.23% APF gel with CAP. Fluoride and CAP were applied to the samples 4 times at 1-wk intervals. The specimens were also stored in artificial saliva for 4 wk to evaluate the retention of fluoride. The fluoride content on the fluoride-treated enamel was measured by an electron probe microanalyzer. To detect the resistance to demineralization, the calcium-to-phosphate ratio of the enamel samples was measured after the application of APF gel with or without CAP, followed by soaking in the demineralization solution. In groups APF1 and APF2, the amount of fluoride detected increased depending on the application frequency, and more fluoride was detected in group APF2 than in group APF1. In the experiment examining the maintenance effect, fluoride was not detected in group APF1, whereas fluoride was detected in group APF2 up to the fourth week. As for the resistance to demineralization, the calcium-to-phosphate ratio of the enamel treated with APF and CAP was higher than that treated with APF alone, and it increased with the frequency of treatment. This study suggests that the combination treatment of CAP and fluoride improves retention of fluoride on the enamel and resistance to demineralization when compared with treatment with fluoride alone.

Keywords: dental care, dental caries, fluoridation, plasma gases, preventive dentistry, tooth demineralization

Introduction

Dental caries is a chronic, multifactorial, and infectious disease that is induced by bacterial acid. While the incidence of dental caries is decreasing because it is preventable, it is still a major public health concern. In the early stages of dental caries, bacteria metabolize carbohydrates, decreasing the pH level below the critical value; consequently, minerals of the enamel are dissolved (Featherstone 2008). Therefore, to prevent dental caries, it is important to increase the resistance against acid, for which calcium (Ca) and phosphate (P) were efficiently adsorbed onto the surface of the enamel (Longbottom et al. 2009).

Fluoride enhances remineralization of the enamel, prevents dental caries by antibacterial function, and provides resistance against erosion (Hicks et al. 2004). There are several methods of applying fluoride to the tooth surfaces. Products with high fluoride concentration, such as 2% sodium fluoride (NaF) solution and 1.23% acidulated P fluoride (APF) gel, are used by professionals in dental clinics (Harris and Garcia-Godoy 2004). The 2% NaF solution is used by applying the product directly to the tooth surface or by performing iontophoresis (Singal et al. 2005); the 1.23% APF gel is applied with a tray or cotton balls. Fluoride varnish was recently used for convenience in application (Güclü et al. 2016).

In the oral cavity, the presence of fluorides decreases the development/progression of dental caries by several mechanisms, such as inhibition of demineralization of the enamel, an

increase in remineralization of the enamel, and inhibition of the bacterial enzyme producers of acids (Paula et al. 2017). On the tooth surface, fluoride forms Ca fluoride-like deposits that increases the hardness of the tooth and its acid resistance by improving the grid structure of the enamel (Dijkman and Arends 1988). After fluoride application, however, a large amount of Ca fluoride-like deposits is washed out of the mouth within a short period. Therefore, fluoride needs to be reapplied

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Figure 1. Schematic diagram of the low-frequency helium plasma device.

at 1-wk intervals (Dijkman et al. 1983). The deposits have low nano-hardness and large nano-wear depth, leading to early loss of Ca fluoride-like deposits after topical fluoride application (Jeng et al. 2008). Although professional fluoride application is known to prevent the progression of caries, there are few effective fluoride products and methods for the mineralization of initial caries lesions (Lee et al. 2010). Therefore, new techniques are needed to enhance the coating effect of fluoride and maintain it for long periods.

Plasma, which is considered the fourth state of matter, is a highly active state that contains many radicals, energetic ions, free electrons, strong electric fields, and charged particles. Furthermore, biomedical applications of plasma—such as blood coagulation (Postgate et al. 2007), bacteria sterilization (Roth et al. 2010), wound healing (Nastuta et al. 2011), and tooth bleaching (Lee et al. 2009)—have been actively studied because plasma is nonthermal and nontoxic. The strong energy of plasma (Laroussi and Lu 2005) was hypothesized to separate fluoride from fluoride compounds, allowing fluoride to replace the hydroxyl group of the hydroxyapatite in the enamel.

In light of this research, this study examined the effect of fluoride application, its retention, and acid resistance of human tooth enamel treated with cold atmospheric plasma (CAP).

Materials and Methods

Preparation of Enamel Specimens

Extracted human molars were used in this experiment. Teeth with dental caries, dental fluorosis, pitting, and cracks were excluded. All suitable teeth were cleaned thoroughly and washed under running water with a scaler to remove all adherent soft tissues, calculus, and stains. The teeth were stored in a 0.4% sodium azide solution (Sigma-Aldrich) to prevent fungal and bacterial growth. Two hundred and sixty enamel specimens were prepared by cutting the teeth into blocks measuring $3 \times 5 \times 1$ mm, with a water-cooled diamond saw (Minitom; Struers) and stored in distilled water prior to use. The 260 specimens were used to investigate the fluoride uptake according to application times and retention with CAP and were divided

randomly into 2 groups (groups A1 and A2; $n = 80$ for each group). Another 90 enamel specimens were used to investigate the resistance to demineralization, and 10 specimens were used for control group (group C). The 80 specimens were divided randomly into 2 groups (groups F1 and F2; $n = 40$ for each group). The study protocol was reviewed by the Institutional Review Board (PNUDH-2014-026) of Pusan National University Hospital.

CAP Device

The plasma jet (Fig. 1) included a dielectric tube (Teflon; radius = 2.3) surrounded by 2 aluminum electrodes: 1 was powered by a low-frequency (20 kHz) sinusoidal high-voltage source (Ramsey power supply), and 1 was grounded. A floating inner electrode (aluminum) was inserted into the dielectric tube between the power and ground electrode. Helium gas, which was regulated by a mass flow meter, was flowed into the dielectric tube and propelled into ambient air. With 2 standard liters per minute of helium gas and an applied voltage of 4 kV, plasma was ignited inside the dielectric tube near the powered electrode. The generated plasma formed a plasma plume outside the electrode for a distance of up to 10 mm (Lee et al. 2011). We earlier demonstrated that the temperature of CAP was around room temperature (Lee et al. 2009).

Fluoride Treatment

The fluoride preparations used was 1.23% APF gel (60 Second Taste Gel; Pascal). In group A1, only 1.23% APF gel was applied; group A2 was treated with 1.23% APF gel and CAP. The application time for all groups was 4 min. After the fluoride treatment for 4 min, residual fluoride compound on the enamel specimens was removed with a cotton swab, rinsed with distilled water, and immersed into artificial saliva (Taliva; Hanlim) containing 1 mg/mL of Na carboxymethylcellulose, 3 mg/mL of D-sorbitol, 0.84 mg/mL of NaCl, 1.2 mg/mL of KCl, 0.15 mg/mL of CaCl_2 , 0.05 mg/mL of MgCl_2 , and 0.34 mg/mL of K_2HPO_4 . Eighty specimens of each group were divided into 8 subgroups, each with 10 specimens, to examine the fluoride uptake effect according to the application number (subgroups S1 to S4) and maintenance effect (subgroups S5 to S8). In subgroups S1 to S4, fluoride was applied 1, 2, 3, and 4 times, respectively, at 1-wk intervals. The specimens were immersed in artificial saliva during the weeklong intervals. In subgroups S5 to S8, fluoride was applied 4 times, and the specimens were immersed in artificial saliva for 1, 2, 3, and 4 wk, respectively. Every time fluoride was applied, the artificial saliva was replaced. The artificial saliva was changed once a day (Fig. 2).

Acid Resistance

Ten teeth samples of groups F1 and F2 were treated with APF and CAP 1, 2, 3, and 4 times at a week interval: group C, control group (no treatment); group F1, 1.23% APF gel alone; group F2, 1.23% APF gel with CAP. Then the samples were

stored in acidified hydroxyethyl cellulose (pH = 4.5) as a demineralization solution for 3 d at 37 °C, and it was changed in every 24 h. After 3 d, samples were washed by distilled water.

Fluoride Analysis

The specimens were dried before analysis. The fluoride uptake effect of the enamel surface was measured by electron probe microanalyzer (SX100; CAMECA). The enamel specimens were coated with carbon for the electron probe microanalyzer. The weight percentage of fluoride on the enamel surface was measured with 4 points under a beam size of 10 μ m, an accelerating voltage of 15 keV, and a beam current of 20 nA.

Ca/P Ratio Analysis

The specimens were dried before analysis. Energy-dispersive X-ray spectroscopy (Supra40 VP; Carl Zeiss) was performed to quantify Ca and P on the enamel specimens. Element analysis through a line scan of the treated region was performed to measure Ca and P content at an accelerating voltage of 15 kV and a magnification of 40 $\times$. The Ca/P ratio was calculated through Ca and P content by line scan.

Statistical Analysis

The collected values were analyzed statistically with SPSS (PASW Statistics 18; SPSS Inc.). The data from ≥ 3 groups and the comparison among the subgroups were assessed with 1-way analysis of variance after verifying normal distribution, followed by Tukey's test. A comparison of groups A1 and A2 was performed with Student's *t* test. The level of significance was set to 95% ($P < 0.05$), and the *P* values were adjusted for multiple testing.

Results

In groups A1 and A2, the fluoride content increased as the number of the fluoride applications increased; the fluoride content of group A2 (subgroup S1 = 23.59, S2 = 26.14, S3 = 30.61, S4 = 35.18; $P < 0.05$) was higher than that of group A1 (subgroup S1 = 13.87, S2 = 14.56, S3 = 22.35, S4 = 25.3; $P < 0.05$; Fig. 3). In the experiment to examine the retention after fluoride application 4 times (subgroups S5 to S8), fluoride was not detected in the A1 groups. In the A groups, the fluoride content of group A2 (subgroup S5 = 12.08, S6 = 7.45, S7 = 1.14, S8 = 0.48; $P < 0.05$) was higher than that of group A1 (subgroups S5, S6, S7, S8 = 0; $P < 0.05$; Fig. 4). Fluoride was not detected in group A1 during 4 wk after fluoride treatment. In contrast, fluoride was detected in group A2.

Figure 2. Schematic diagram of the study design. The duration of each cycle was a week, and the specimens were immersed in artificial saliva during each cycle. EPMA, electron probe microanalyzer.

Figure 3. Mean fluoride content on the enamel surface according to the treatment times in the A groups. * $P < 0.05$. Error bars indicate standard error.

After confirmation of fluoride application to the enamel, we analyzed the Ca/P ratio to investigate the resistance of the enamel to demineralization. In group F2 (treated with CAP and fluoride), a higher Ca/P ratio was shown. In particular, statistically significant differences were observed when treated 3 or 4 times. In the first application, the Ca/P ratios of groups C, F1, and F2 were 1.8, 1.76, and 1.83, respectively. On treating 2 times, the Ca/P ratios of groups F1 and F2 were 1.76 and 2.06, respectively. On treating 3 times, the Ca/P ratios of groups F1 and F2 were 1.84 and 2.36, respectively. On treating 4 times, the Ca/P ratios of groups F1 and F2 were 1.94 and 2.82, respectively (Fig. 4).

Discussion

This study revealed the fluoride uptake by the tooth surface treated with CAP. The formation of Ca fluoride after topical

Figure 4. The calcium-to-phosphate (Ca/P) ratio of the enamel according to the treatment times. * $P < 0.05$. Error bars indicate standard error.

fluoride application reduces the acid solubility of the enamel surface (Wiegand and Attin 2003). A range of commercial fluoride products is used to prevent dental caries and inhibit tooth hypersensitivity.

Fluoride was detected when 1.23% APF gel was applied to the enamel specimens. Furthermore, its amount increased on treating with a combination of CAP and 1.23% APF gel. This suggests that the Ca combined with a large amount of fluoride released from the fluoride compounds due to the CAP treatment. Fluoride reduces the permeability and fluid movement of the enamel and enhances remineralization (Robinson et al. 2000). Enamel permeability has been reported to be related to the pH of fluoride (Takagi et al. 1987). The acidic fluoride enhances mineralization of the noncavitated carious lesions and increases the fluoride uptake (González-Cabezas et al. 2012). In addition, a study showed that the effect of permeability reduction was maintained when APF gel was applied (Chersoni et al. 2011).

In the experiment of resistance to demineralization, energy-dispersive X-ray spectroscopy data showed that the Ca/P ratio of group F2 (treated with CAP and fluoride) was higher than those of the control and F1 groups. The Ca/P ratio of group F2 was increased with increasing treatment frequencies. This demonstrates that fluoride plays a role in the resistance to demineralization, and the efficiency of this process can be enhanced by CAP treatment. Fluoride interacts with hydroxyapatite to form fluorapatite, which is resistant to acid, thereby preventing hydroxyapatite from dissolution (Hicks et al. 2004). In addition, Ca fluoride contributes in preventing dental caries: Ca fluoride inhibits the dissolution of minerals from the enamel surface and maintains a high concentration of fluoride in the oral cavity, accelerating the remineralization of the enamel (Hicks et al. 2004; Chu et al. 2010). This effect of fluoride was confirmed in this experiment; thus, effective application of

fluoride on the enamel is very important to induce the remineralization of the enamel. Therefore, we find fluoride application with CAP highly effective for remineralizing the enamel.

Many studies combining various techniques (e.g., laser) are under active discussion. Anderson et al. (2000) reported that resistance against dental caries increased when an argon laser was used with 2% NaF solution. The argon laser alters the surface layer of the enamel by producing a globular surface coating and mixed microporosity. This surface has abundant fluoride, Ca, and phosphorus, which enhance the resistance to dental caries (Westerman et al. 1996). Rios et al. (2009) indicated that the application of APF gel with a Nd:YAG laser may be the key to prevent dental erosion. Moreover, Altinok et al. (2011) reported that Er:YAG laser irradiation alone can reduce enamel solubility, although synergy effects were not observed in case of the laser treatment with APF gel. Fluoride application with a laser had a different effect according to the types of lasers and fluoride products. High-intensity irradiation is required to achieve the desired effect; thus, the risk of damaging the underlying pulp increases (Jeng et al. 2013). In addition, a laser has linearity; therefore, it has some limitations when applied to the oral tissues with an irregular structure. As such, the clinical results of fluoride application with laser irradiation are still controversial. In contrast, plasma is a form of low-temperature ionized gas. Therefore, its high degree of accessibility acts as an advantage when treating complex oral tissues. Moreover, it causes no damage to the tissues.

The results showed that the effects of CAP treatment with fluoride were maintained for a longer duration when compared with the effects of only fluoride treatment. This suggests that CAP has a higher probability of producing negative fluoride ions. Because the concentration of negative fluoride ions is an important factor for a fluoride application, it can be postulated that CAP showed a result that was maintained long-term. The existence of high-energy electrons implies a higher concentration of reactive species and more interactions among the particles. Moreover, hydroxyl radicals, which are a reactive oxygen species, interact with the surface of the tooth and can alter the surface characteristics, including hydrophilicity and hydrophobicity (Kang et al. 2012). The change in surface characteristics can influence the efficacy of the fluoride treatment.

The equilibrium constants of hydroxyapatite and fluorapatite at 25 °C are as follows:

Therefore, the equilibrium constant of the reaction that replaces the hydroxide ion with a fluoride ion is 7.2 at 25 °C:

As the equilibrium constant ($K = 7.2$) derived on the basis of formula 3 is relatively large, the reaction rate becomes slow. Owing to the demand of high activation energy for this reaction, it is difficult to form fluorapatite on the enamel surface

during general dental treatment. It is thought that CAP treatment provides energy exceeding the activation energy required according to formula 3 so that fluorapatite would be easily formed on the surface of tooth enamel. However, this potential mechanism needs to be verified chemically.

According to the limited results of this study, fluoride application with CAP would be considerably more effective than fluoride application without CAP in terms of uptake and retention of fluoride to the enamel and resistance to demineralization.

Author Contributions

Y.M. Kim, contributed to design, data acquisition, analysis, and interpretation, drafted the manuscript; H.Y. Lee, contributed to data acquisition and analysis, drafted the manuscript; H.J. Lee, contributed to conception and design, critically revised the manuscript; J.B. Kim, S. Kim, contributed to conception, critically revised the manuscript; J.Y. Joo, G.C. Kim, contributed to conception, design, and data interpretation, critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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