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Change in the Salivary Fluoride Concentration through Nuclear Magnetic Resonance Spectroscopy

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Fluoride application has an excellent effect for the prevention of dental caries, and 1.23 % acidulated phosphate fluoride (APF) gel is the most commonly used fluoride formulation for professional fluoride application. There has been a problem, however: its excessive intake may cause toxicity in the human body. The purpose of this study was to evaluate the amount of residual fluoride present in the saliva in the mouth over time by measuring it with ^{19}F NMR spectroscopy after the topical application of 1.23 % APF gel. After 30-minute application, a very small amount of fluoride (0.000482 %) remained in the saliva in the mouth, indicating that there was no effect on the human body. Therefore, this study demonstrated that clinical fluoride application using 1.23 % APF gel is safe.

Keywords : nuclear magnetic resonance (NMR) spectroscopy, fluoride, saliva, acidulated phosphate fluoride (APF) gel

1. Introduction

The outer covering of the tooth is known as the enamel. It is the hardest tissue in the human body because it contains almost no water. During the mastication of food, the enamel comes in direct contact with the chemicals and acids present in the food [1].

Dental caries is the most common chronic dental disease, and the early caries lesion is the site that has been demineralized due to mineral loss. It appears even whiter than the normal enamel as the minerals have escaped from its surface [2]. It is often neglected, however, and develops into a deeper caries lesion. Therefore, early enamel caries can be remineralized without restoration if the proper environment is established, so it is very important to detect lesions and perform appropriate treatment at this stage [3].

The most typical and widely used method of remineralizing early caries and preventing dental caries is using fluoride, whose effect has been proven through many clinical studies [4]. The method of preventing dental caries using fluoride is divided into systemic application and topical application. Between the two, it is topical application that has been utilized to date by experts in the field of dentistry since its introduction by Knuson and Armstrong [5] in the early 1940s.

Fluoride application is an effective method of promoting remineralization and inhibiting demineralization [6], and acidulated phosphate fluoride (APF) gel is the most commonly used method in clinical practice. It is a formulation made by acidulating a 2-3 % sodium fluoride (NaF) solution with phosphoric acid, which is relatively safe and commonly used for topical application in clinical practice [7]. APF gel has been recognized to be easily absorbed by the enamel and to have good access between teeth and to the fine parts of the teeth [8, 9]. It is safe when used properly, and can be effectively used to prevent and control caries. Children, however, may swallow fluoride because it is difficult for them to control their behavior during topical fluoride application, which has been pointed out as a problem [10]. Additionally, the possibility that APF gel has systemic toxicity has been reported, along with the possibility that it will cause discomfort, such as temporary nausea and vomiting, when ingested during a 4-minute application time. Attention should be paid to these concerns [11].

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Nuclear magnetic resonance (NMR) spectroscopy is one of the most powerful analytical techniques whose application scope continues to expand. It has gained widespread acceptance of late as a most versatile tool [12, 13]. In addition, quantitative analytical application has become an important non-invasive approach employed in many fields, including foods, beverages [14, 15], and pharmaceuticals [16]. This is because the NMR methods have been proven to be simple, reasonably rapid, and cost-effective in the long term [17]. As a basis for the quantitative determination of fluorinated species, fluorine (^{19}F) NMR is a powerful, universal, and fast-screening technique for evaluating the quality and quantity of drugs. Its important advantage is that the resonances of the fluorine nuclei can be observed without interfering background signals because the level of endogenous fluorine-containing compounds is very low [18].

Therefore, the aim of this study was to validate and apply a ^{19}F NMR spectroscopic method for determining the fluorine content of saliva after APF gel application.

2. Materials and Methods

2.1. Sample preparation

In the experiment in this study, 1.23 % APF gel (60 seconds taste®, Pascal, Belleuve, USA) was used as a fluoride formulation. A total of 6 ml APF gel was evenly placed on the maxillary and mandibular tray and was made to contact the teeth in the mouth for 4 minutes, and then removed. The subject was told to spit out his saliva and not to swallow it in a span of 30 minutes, according to the manufacturer's instructions. The saliva in the subject's mouth was collected 5, 10, 20, and 30 minutes after fluoride application.

2.2. NMR measurement

The ^{19}F NMR measurements were carried out on an ECZR NMR spectrometer (FT-NMR 400 MHz Spectrometer, JNM-ECZ400S/L1, JEOL Ltd., Tokyo, Japan) operating at 376.17 MHz and equipped with a dedicated 5mm spinning probe (Fig. 1). The probe temperature was 23°C. The typical spectral parameters for this study were as follows: 90° pulse width, 6.74 μs; relaxation delay, 5 s; and acquisition time, 83.88 s. A known amount of D_2O (100 μl) was added as an internal field frequency lock.

The nuclear magnetic resonance spectrometer were calculated using the following formula:Spectral resonance frequency ($V0$)

$$V0 = \frac{\gamma}{2\pi} B0$$

Fig. 1. (Color online) 400 MHz FT-NMR spectrometer used in this study.

r = gyromagnetic ratioq
$B0$ = Magnetic Field strength

And Chemical shift (ppm) were used the following fomula.

$$\text{Chemical shift (ppm)} = \frac{Vi - V^{ref}}{V0} \times 1{,}000{,}000$$

$V0$ = resonance frequency of the non-nuclear chemical bonds
Vi = resonance frequency of each element in the molecule
V^{ref} = reference frequency

3. Results

3.1. Fluoride analysis via ^{19}F NMR

The analysis of the ^{19}F NMR spectrum showed a −122.44 to −120.41 ppm range (Fig. 2). These peaks represented the amount of fluoride residue in the oral cavity. The decrease in the total peak areas indicated that the fluoride concentration in the saliva after APF gel application decreased over time (Fig. 3). Even after only 5-minute fluoride application, only 4.66 % fluoride residue was measured in the mouth. After 30 minutes, there was almost no fluoride residue in the mouth (0.000482 %).

4. Discussion

Fluoride has been known to be deposited on the tooth surface and to make the enamel harder and less soluble to

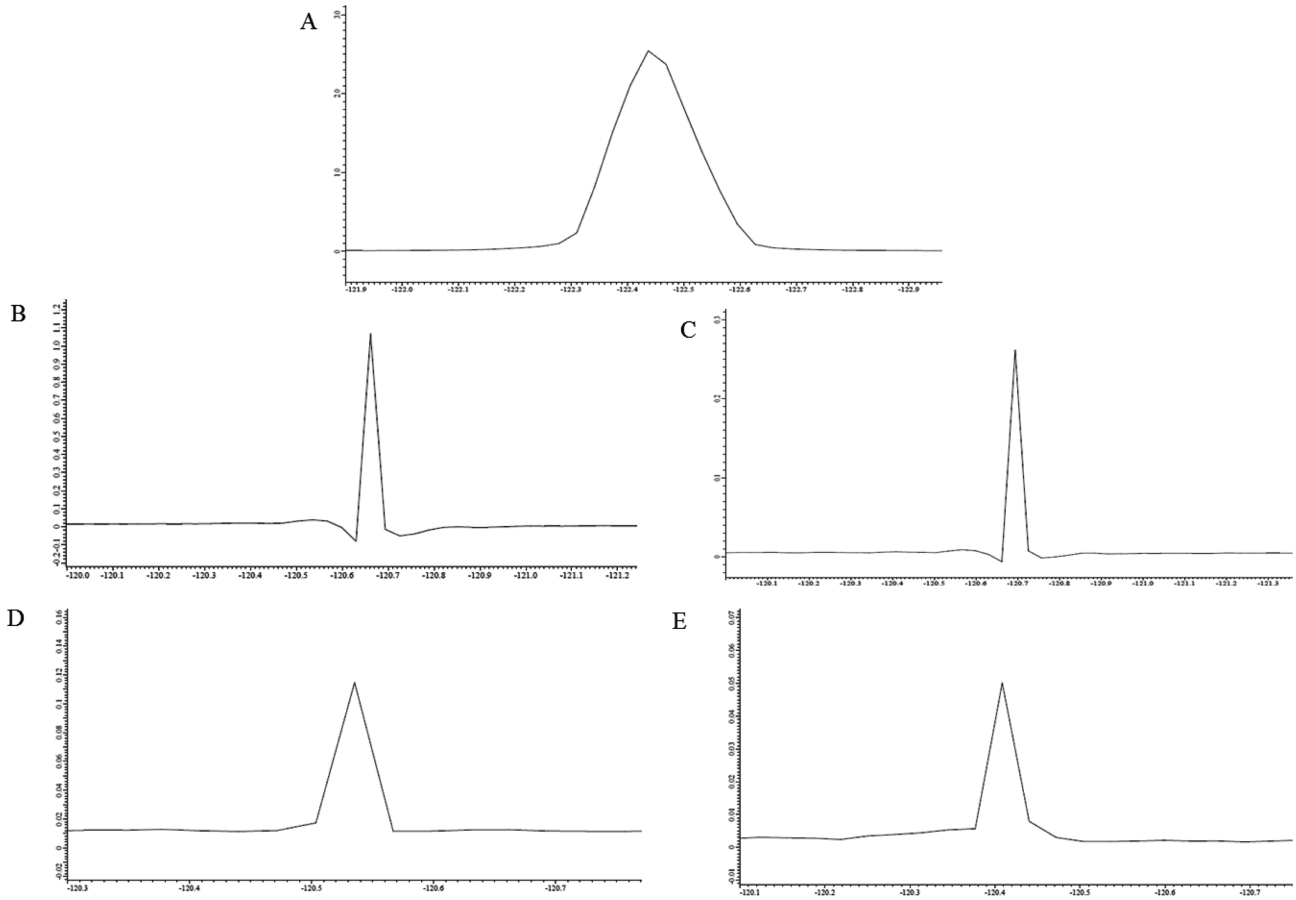

Fig. 2. Fluoride residue in the saliva. ^{19}F NMR spectra: (A) 1.23 % APF gel; (B) after 5-minute fluoride application; (C) after 10-minute fluoride application; (D) after 20-minute fluoride application; and (E) after 30-minute fluoride application.

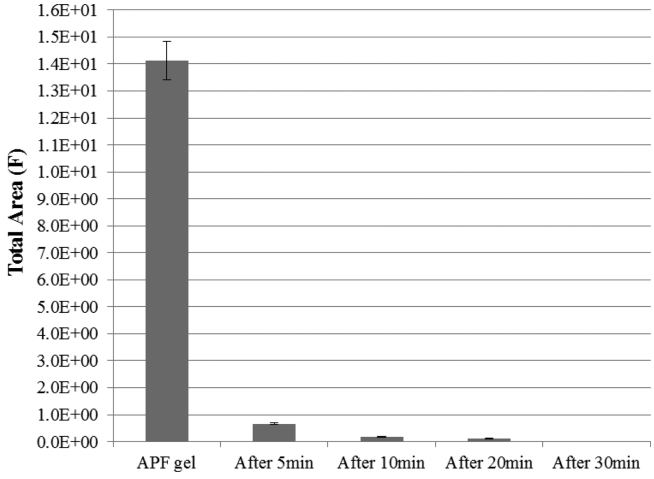

Fig. 3. Mean salivary fluoride concentration levels as determined at the total peak area after fluoride application using ^{19}F NMR.

acid as well as to improve the acid resistance of the tooth structure or promote the remineralization of the demineralized tooth structure, thereby preventing dental caries [19]. APF gel, a topical fluoride supply method, has been reported to have a higher fluoride absorption effect in the enamel and a higher clinical caries inhibition effect than neutral NaF [20]. On the other hand, APF gel requires a relatively long application time (4 minutes), and there is a concern about excessive swallowing during the application process [21]. In fact, it has been reported in a study that 14-31 mg fluoride is swallowed upon a single topical application of 1.23 % APF gel [22]. As excessive intake may cause toxicity, its use in pediatric patients is inevitably limited. There is still limited research, however, on the precise criteria for measuring the residual fluoride in the mouth [23]. In this study, the amount of residual fluoride in the mouth after the application of 1.23 % APF gel was measured and evaluated over time through the saliva, for safe and effective topical fluoride application.

Fluoride is present in the saliva in the ionic and bonded forms, but much of the fluoride in the saliva is in ionic form, and the fluoride present in bonded form is easily ionized [24]. Among the various methods of measuring the fluoride concentration, the method using a fluoride ion electrode has been widely applied of late [25]. The

fluoride ion electrode reacts with the fluoride ion that is not bound to the compound, and reacts to the ion activity rather than the concentration itself, necessitating buffering at an appropriate pH level [26]. In addition, as the measurement limit of the electrode is about 10^{-6} M (0.02 ppm fluoride), the process of enriching and diffusing the fluoride must have been completed when the sample is measured at a concentration lower than this, which causes measurement errors and inconvenience [27].

NMR spectroscopy offers unparalleled rich information on samples, and when used with other rapid validated methods, high throughput can be achieved without destroying the sample [28]. A number of previously published study results revealed that ^{19}F NMR spectroscopy could be employed as a powerful selective tool for analyzing different fluorinated drugs [29]. As the fluorine atom has a smaller atomic radius and stronger electronegativity than other atoms due to the chemical nature of fluoride, research using NMR has been carried out, but most of the studies have been on structure analysis and efficient synthesis [30, 31]. Applied research using NMR to analyze fluoride substituted into crystal structures has been carried out [32], but there have been no studies that used NMR to analyze the residual fluoride in the saliva after the application of fluoride to the surfaces of the teeth in the mouth. Therefore, in this study, the amount of residual fluoride in the saliva was measured using NMR spectroscopy after the application of 0.23 % APF gel in the mouth.

After 5-minute application of 1.23 % APF gel, the initial fluoride concentration decrease was 95.34 %, and the amount of residual fluoride in the mouth was 4.66 %. As a result of the continuous concentration reduction due to dilution by the new saliva, it was found that only a small amount of fluoride (0.000482 %) remained after 30-minute APF gel application. Therefore, it was confirmed that even if the residual fluoride in the saliva was swallowed, it had no effect on the human body, such as toxicity. The above study results demonstrate that fluoride application is an effective dental caries prevention method when a proper fluoride concentration is used, and that as the fluoride content of the saliva in the mouth after fluoride application is very small, it is an easy and safe clinical procedure.

5. Conclusions

This study provided evidence for the safety of fluoride application as a dental caries prevention method as the amount of residual fluoride in the saliva in the mouth after fluoride application was very small. As the precise fluoride concentration in the saliva after 30-minute fluoride application was found to be very small, it is unlikely for patients to be exposed to the side effects of fluoride after its application, and fluoride application can be said to be an ideal dental caries prevention method.

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