ISSN (Print) 1226-1750
ISSN (Online) 2233-6656
Journal of Magnetics 28(4), 456-461 (2023) https://doi.org/10.4283/JMAG.2023.28.4.456

Residual Analysis of Salivary Fluoride Concentration after Rinsing with Fluoride-containing Mouthwash Using Nuclear Magnetic Resonance Spectroscopy

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(Received 23 October 2023, Received in final form 29 November 2023, Accepted 5 December 2023)

Fluoride compounds are major substances that inhibit the growth of oral bacteria, increase the hardness of the tooth surface, and promote recalcification. However, caution must be exercised regarding fluoride exposure, as it may cause side effects to the human body due to its toxicity if swallowed. Accordingly, this study was conducted to evaluate the residual amount of fluoride remaining in saliva after using mouthwashes containing high and low fluoride concentrations. After rinsing with five commercially available high-fluoride mouthwashes according to the manufacturer's instructions, the fluoride concentration remaining in saliva was quantitatively evaluated over time through Fluorine (^{19}F) NMR (magnetic field: 9.4 Tesla) immediately, 1 minute, and 2 minutes later. When gargling with DH 2000 ppm fluoride, the residual salivary fluoride content was 30.3960 % immediately after gargling, 0.0041 % after 1 minute, and 0.0020 % (0.0401 ppm) after 2 minutes; when gargling with EB 1000 ppm, it was 16.5001 % immediately after gargling, 10.6269 % after 1 minute, and 0.0034 % after 2 minutes; and when gargling with BS 1000 ppm, it was 17.1169 % immediately after gargling, 13.2337% after 1 minute, and 0.0019 % (0.0188 ppm) after 2 minutes. When gargling with LIS 220 ppm, the residual salivary fluoride content was 56.3716 % immediately after gargling, 0.0842 % after 1 minute, and 0.0180 % (0.0396 ppm) after 2 minutes; and when gargling with R 90 ppm, it was 0.0302 % immediately after gargling, 0.0151 % after 1 minute, and 0.0077 % (0.0069 ppm) after 2 minutes. Even when mouthwashes containing various fluoride concentrations were used, only the fluoride content of existing normal saliva remained after 2 minutes, confirming that swallowing the saliva is safe and does not affect the human body.

Keywords : Nuclear magnetic resonance (NMR) spectroscopy, magnetic fields; fluoride-containing mouthwashes, residual fluorine concentration, saliva, gargling

1. Introduction

Dental caries and dental erosion are major diseases that damage the hard tissues of teeth. Dental caries refers to damage to the hard tissues of teeth caused by acids produced by bacteria in the oral cavity, while dental erosion refers to the loss of the hard tissues of teeth caused by chemical actions regardless of bacteria [1].

Fluoride is absorbed into demineralized crystals and promotes the absorption of calcium and phosphorus, and fluoride deposited on the tooth surface is known to inhibit demineralization caused by acids formed in dental plaque, promote enamel remineralization, and improve acid resistance [2, 3]. Using fluoride is one of the widely used methods of preventing dental caries and dental erosion or minimizing damage due to caries inhibition and recalcification properties [4]. For this reason, fluoride is mixed and used in auxiliary products that manage the oral environment, such as toothpaste and mouthwash.

Among them, the fluoride-based mouthwash method has been developed and marketed in many countries. This method has become prevalent due to the advantage and ease of purchasing such quasi-drugs available in supermarkets and convenience stores. Moreover, using fluoride does not require professional skills, and is convenient to use, and carry [5, 6]. The use of fluoride-containing mouthwash is recommended in patients with caries and erosion lesions, orthodontic appliances or prostheses, gingival recession, chemotherapy or radiotherapy, reduced salivary flow, and physical or mental disorders [7]. The representative fluoride compound contained in commercially available mouthwashes is sodium fluoride (NaF),

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which has been widely used around the world after its efficacy was proven [8].

There are advantages when fluoride is consumed in an appropriate amount (0.8-1.2 ppm), but if consumed over a certain amount every day for a long period, it can be toxic to nervous system tissues and may affect brain development [9], and causes a lot of damage to animal and human organs such as the liver, kidney, and spinal cord, as well as mainly to the skeletal system [10, 11]. Fluoride requires great caution as susceptibility to even small amounts of fluoride can be high. Excessive intake can cause nausea, vomiting, and toxicity, and the amount that can be used is limited due to concerns about side effects such as mottled tooth [12]. Therefore, to avoid the risk of fluoride toxicity and ensure the effectiveness of mouthwash, it is necessary to check the release of fluoride over time after using various fluoride-containing mouthwashes and to know the amount of fluoride remaining in the oral cavity.

Saliva dilutes and removes erosive substances in the oral cavity, neutralizes and buffers acids, reduces the rate of enamel dissolution through the ionic effect of calcium and phosphorus in saliva, and forms an acquired pellicle [13]. As inorganic components in saliva, the most abundant ions are sodium and potassium, the fluoride concentration is approximately 1 μmol/L (0.07 ppm), and fluoride is known to be present in the oral cavity throughout one's life [14]. However, the exact amount and measurement standards are not yet sufficient to measure fluoride remaining in the oral cavity [15].

The method of using a fluoride ion electrode to measure the amount of fluoride in the oral cavity requires a diffusion process of fluoride in response to the activity of the ion rather than the concentration itself. Measurement using ion chromatography reacts with fluoride in an ionic state, and when NaF is contained, the concentration is measured to be high because ions are separated well [16]. On the other hand, Nuclear Magnetic Resonance (NMR) Spectroscopy analyzes the structure of materials using the magnetism of the nucleus, with an analytical device that performs molecular structure analysis and composition analysis of compounds. It is non-destructive, and dynamic information can be obtained. This NMR method is used to analyze the components of samples and obtain high-resolution micro-images and non-destructive tomographic images of the human body for medical purposes. In addition, in terms of quality control, it is also used to examine the characteristics of produced products [17-19]. Accordingly, this study analyzed fluoride using ^{19}F NMR for quantitative evaluation of fluoride, which is sensitive to measurement [20].

Therefore, this study compared and analyzed the residual fluoride concentration in saliva with ^{19}F NMR measurements after using commercially available mouthwashes containing various concentrations of fluoride.

2. Materials and Methods

2.1. Saliva collection procedure

Five types of commercially available fluoride-containing mouthwashes with an expiration date of more than 6 months were purchased (Table 1). For randomized, blind testing, fluoride-containing mouthwashes were coded, and saliva was collected in sterile containers after the mouthwashes were used, following each manufacturer's instructions. To measure the residual fluoride remaining in saliva over time, 400 μl of saliva collected without swallowing immediately, 1 minute, and 2 minutes after using a fluoride-containing mouthwash was dissolved in Deuterium Oxide (D_2O) and analyzed using NMR. All experiments were performed in triplicate.

Table 1. Different concentrations of commercial fluoride-containing mouthwashes used in this study.

Products	Total fluoride (ppm)	Active ingredients	Manufacturer	Code
Dentiheal Teeth Remineralization Gargle	2000	Sodium fluoride Sodium pyrophosphate	Haelim Dentech	DH
EICA LABS Essential Breath	1000	Sodium fluoride Sodium pyrophosphate	EICA LABS	EB
Median Odor Science Freezing Cool Mint	1000	Sodium monofluorophosphate Sodium pyrophosphate	Amorepacific	BS
Listerine Total Care Plus	220	Sodium fluoride	Johnson & Johnson	LIS
Rucipello Breath-care Mystic Forest Gargle	90	Sodium fluoride	Rucipello	R

2.2. Fluoride measurement using NMR

To detect fluoride, ^{19}F NMR analysis was performed on an ECZR NMR spectrometer (FT-NMR 400 MHz Spectrometer, JNM-ECZ400S/L1, JEOL Ltd, Tokyo, Japan) equipped with a dedicated 5 mm rotating probe and operating at 400 MHz. On the NMR with standard frequency: 400 MHz, pulse controller - time resolution: 5 ns, magnetic field: 9.4 Tesla, and sensitivity: 19 F-500 more, the temperature of the probe mounted was 23 °C. When operating to the saliva collected over time with the spectral parameters of about 1 hour, using 90° pulse width 6.74 μs, relaxation delay 5 seconds, and NMR data scans 62, D_2O (700 μl) solvent was added as a locking agent for locking. The following formula was used to calculate and evaluate the NMR spectrometer using the spectral resonance frequency (V0):

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

where

r = gyromagnetic ratio

B0 = magnetic field strength

Chemical shift (ppm) uses the following formula:

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

where

V0 = the resonance frequency of the chemical bonds that are not nuclear

Vi = the resonance frequency of each element in the molecule

V^{ref} = the reference frequency

2.3. Statistical analysis

The statistical significance was verified with SPSS 24.0 for Windows (IBM Corp., Armonk, NY, USA) at a significance level of 5 %, using one-way ANOVA and Duncan tests as a post-hoc test to evaluate changes over time according to the application of five types of gargles.

3. Results

As a result of the ^{19}F NMR spectrum, the processing range was -119 ~ -130 ppm, and the detection of fluoride was confirmed through the peak (Fig. 1). The results of measuring the amount of fluoride remaining in saliva in the oral cavity were as follows: For the three types of high-fluoride DH mouthwashes at 2000 ppm, the residual salivary fluoride was measured to be 30.3960 % immediately after gargling, 0.0041 % after 1 minute, and 0.0020 % after 2 minutes. For the EB mouthwash at 1000 ppm, the residual salivary fluoride was measured to be 16.5001 % immediately after gargling, 10.6269 % after 1 minute, and 0.0034 % after 2 minutes. For the BS mouthwash at 1000 ppm, the residual salivary fluoride was measured to be 17.1169 % immediately after gargling, 13.2337 % after 1 minute, and 0.0019 % after 2 minutes. For the two low-fluoride types, the residual salivary fluoride concentration was 220 ppm for LIS mouthwash, with 56.3716 % immediately after gargling, 0.0842 % after 1 minute, and 0.0180 % after 2 minutes, while it was 90 ppm for the R mouthwash, with 0.0302 % immediately after gargling, 0.0151 % after 1 minute, and 0.0077 % after 2 minutes (Fig. 2).

The concentrations immediately, 1 minute, and 2 minutes after gargling were 607.9209ppm, 0.0817 ppm, and 0.0401 ppm, respectively, for the DH mouthwash; 165.0009 ppm, 106.2686 ppm, and 0.0340 ppm, respectively, for the EB mouthwash; 171.1686 ppm, 132.3372 ppm, and 0.0188 ppm, respectively, for the BS mouthwash; 124.0176 ppm, 0.1815 ppm, and 0.0396 ppm, respectively, for the LIS mouthwash; and 0.0272 ppm, 0.0136 ppm, and 0.0069 ppm, respectively, for the R mouthwash. Statistically, there was a decrease for DH, LIS, and R between 1 and 2 minutes after applying the gargle, but there was no significant difference ($p>0.05$), and for EB and BS, there was a decrease with a significant difference between 1 and 2 minutes after applying the gargle ($p<0.05$). The results showed that a very small amount of fluoride remained in saliva in the oral cavity 2 minutes after gargling (Table 2).

4. Discussion

Saliva maintains ecological balance through repeated tooth demineralization and remineralization due to protective factors containing fluoride [21]. Fluoride, used as a method to promote remineralization, is the most widely used antibacterial material in the oral health field. Various methods of applying fluoride are supplied, and the easily available mouthwash fluoride products are used as they are capable of chemical plaque control and strengthening crystals of enamel [22]. Recently, as interest in oral care has increased, the types of mouthwash solutions on the market have become more diverse, and they are composed of safe drugs and most widely used [23].

Due to the toxicity of fluoride when ingested excessively, a 3-year-old boy swallowed 4 % stannous fluoride solution after brushing his teeth and immediately vomited but died 3 hours later [24]. As such, the use of fluoride mouthwash is especially limited in children who cannot spit out the mouthwash, and great caution is

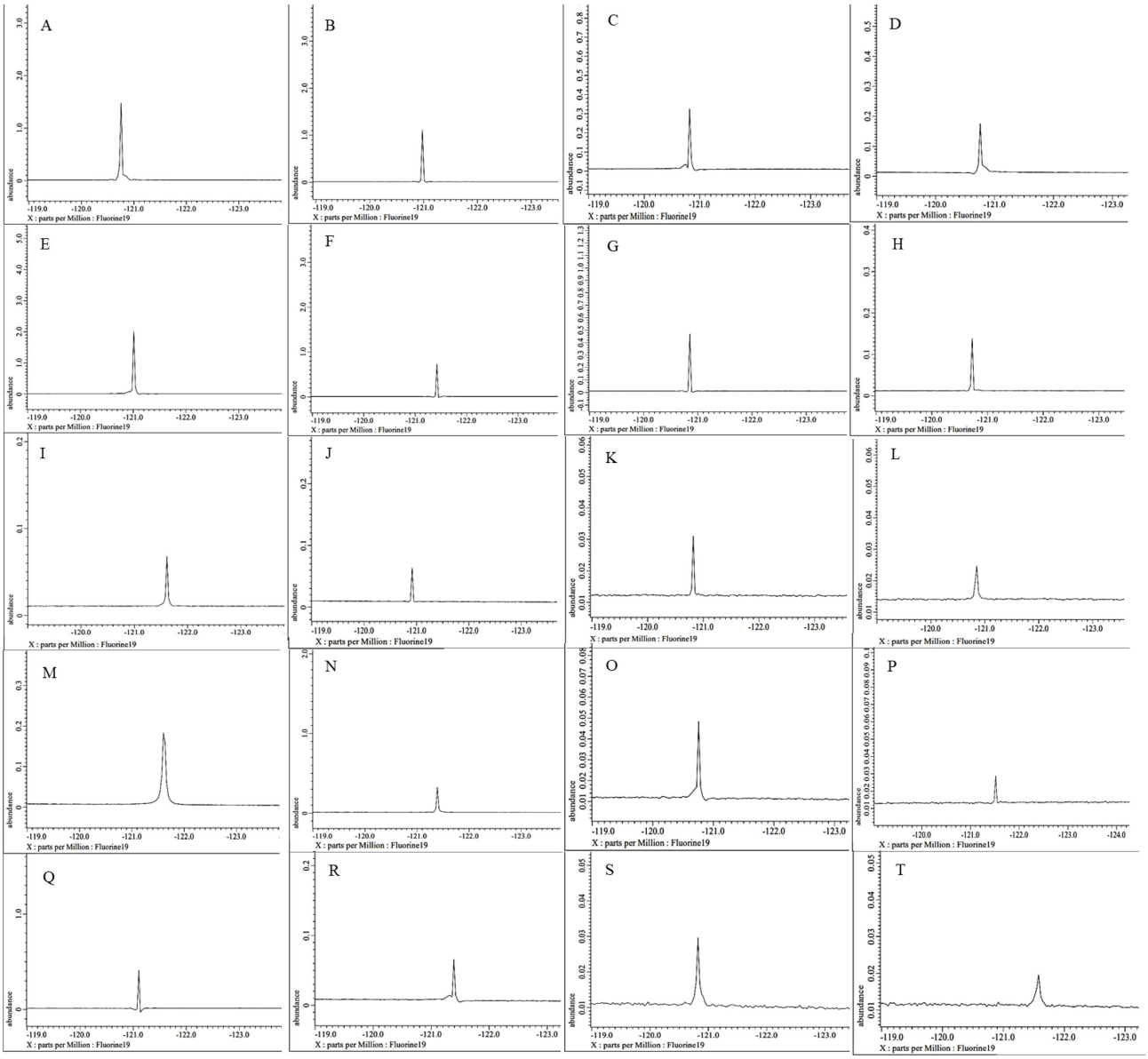

Fig. 1. Peak of residual fluoride concentration in saliva detected through NMR analysis. A: DH 2000 ppm, B: Immediately after gargling with DH, C: 1 min after gargling with DH, D: 2 min after gargling with DH, E: EB 1000 ppm, F: Immediately after gargling with EB, G: 1 min after gargling with EB, H: 2 min after gargling with EB, I: BS 1000 ppm, J: Immediately after gargling with BS, K: 1 min after gargling with BS, L: 1 min after gargling with BS, M: LIS 220 ppm, N: Immediately after gargling with LIS, O: 1 min after gargling with LIS, P: 2 min after gargling with LIS, Q: R 90 ppm, R: Immediately after gargling with R, S: 1 min after gargling with R, T: 1 min after gargling with R

needed as they are highly sensitive to even small amounts of fluoride in some cases. Petechial hemorrhages were observed in the gastric mucosa during an endoscopy 2 hours after application of the fluoride gel [25], and when exposed to fluoride above a certain concentration for a long time, it has been known to cause various oxidative stresses in the body, resulting in increased saturated/ unsaturated fatty acids ratio in the blood and decreased concentration of testosterone, negatively affecting the male reproductive system [26-28]. As such, it has been reported that fluoride can have side effects on the human body if misused [29].

Therefore, research is needed on how long fluoride remains in the oral cavity when it is applied. Commercially available mouthwashes contain fluoride, but recently, only the negative aspects of fluoride have been emphasized, leading to an increase in negative awareness of fluoride-containing products without much awareness

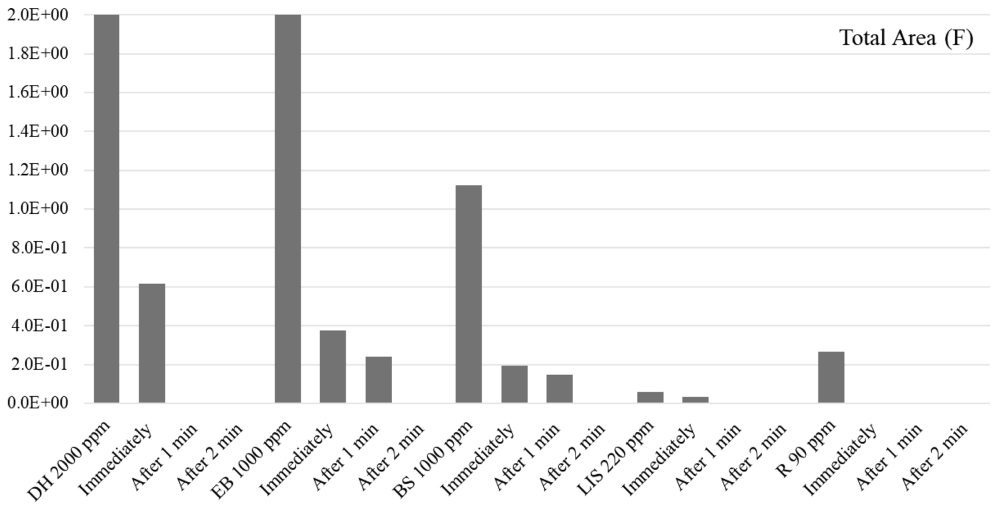

Fig. 2. Changes in residual fluoride concentration in saliva after using various concentrations of fluoride-containing mouthwash.

Table 2. Comparative evaluation of quantified salivary fluoride concentration after using mouthwash.

Products	Group	Total fluoride (ppm)	Residual salivary fluoride (ppm) Mean ± SD	p-value
DH	Immediately	2000[a]	607.9209 ±0.0257[b]	0.000*
	After 1 min		0.0817±0.0148[c]	
	After 2 min		0.0401±0.0121[c]	
EB	Immediately	1000[a]	165.0009±0.0462[b]	0.000*
	After 1 min		106.2686±0.0354[c]	
	After 2 min		0.0340±0.0102[d]	
BS	Immediately	1000[a]	171.1686±0.0531[b]	0.000*
	After 1 min		132.3372±0.0363[c]	
	After 2 min		0.0188±0.0095[d]	
LIS	Immediately	220[a]	124.0176±0.0356[b]	0.000*
	After 1 min		0.1851±0.0127[c]	
	After 2 min		0.0396±0.0076[c]	
R	Immediately	90[a]	0.0722±0.0035[b]	0.000*
	After 1 min		0.0136±0.0032[c]	
	After 2 min		0.0069±0.0016[c]	

*p-values are determined by one-way ANOVA and Duncan tests ($p<0.05$). Different letters (a, b, c, and d) indicate the statistically significant parameters.

of the effects of fluoride. Accordingly, this study was conducted to evaluate concerns about fluoride exposure by checking the residual fluoride concentration in saliva in the oral cavity following the use of mouthwashes with various fluoride concentrations.

NaF supports the recalcification process, but its duration in saliva is short. The bioavailability of fluoride in saliva depends on several factors, including dietary fluoride intake, salivary secretion rate, and use of fluoride products. Fluoride toothpaste or fluoride-containing gum was removed within 2 hours, and for fluoride gel or fluoride mouthwash, fluoride only remained in the oral cavity for about 2 to 24 hours [30]. In this study, 2 minutes after gargling with DH 2000 ppm, which is the highest fluoride concentration available on the market, 0.0020 % (0.0401 ppm) of fluoride remained; 2 minutes after gargling with EB 1000 ppm, 0.0034 % (0.0340 ppm) remained; 2 minutes after gargling with BS 1000 ppm, 0.0019 % (0.0188 ppm) remained; 2 minutes after gargling with LIS 220 ppm, 0.0180 % (0.0396 ppm) remained; and 2 minutes after gargling with R 90 ppm, 0.0077 % (0.0069 ppm) remained. Two minutes after applying the high-concentration fluoride-containing mouthwash and the low-concentration fluoride-containing

mouthwash, the amount of residual salivary fluoride in the oral cavity was very small and similar to the average concentration of fluoride in oral saliva at a safe concentration without the risk of fluoride exposure. They were safe concentrations within the range of a study reporting that oral saliva has a fluoride content of about 0.1 ppm [31] and a study reporting that the average fluoride content in saliva is 0.02-0.05 ppm [32]. However, caution is needed as more than 10 % of fluoride was exposed 1 minute after application of the two types of 1000 ppm mouthwashes, with EB at 10.6269 % and BS at 13.2337 %. Therefore, when using a fluoride-containing mouthwash, there is a risk of swallowing fluoride, so it is recommended that you spit out the saliva instead of swallowing it for 2 minutes after applying the mouthwash. After 2 minutes, it was confirmed that even if the remaining fluoride in the saliva was swallowed, it was harmless because it was the fluoride concentration present in existing saliva. In future research, it is believed that additional research will be needed to measure fluoride concentrations using more accurate and diverse conditions and apply various analysis methods for comparative analysis.

5. Conclusions

When using mouthwashes containing various fluoride concentrations, swallowing may occur due to carelessness, and fluoride may remain in the oral cavity. Thus, for safe use, the residual amount of fluoride remaining in saliva was analyzed through ^{19}F NMR. Even if it is a mouthwash containing a high fluoride concentration, only a very small amount of fluoride remains in the oral cavity 2 minutes after gargling without fear of fluoride exposure, hence, confirming that using fluoride-containing mouthwashes is a safe oral health care method.

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