

HMU Fluorinze Mouthwash Enhances Enamel Remineralization: An In Vitro Study

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Abstract

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BACKGROUND: Fluoride therapy has long been used extensively to prevent dental caries. Fluoride appears in a variety of dental care products such as mouth rinses, dentifrices, gels, etc. HMU Fluorinze is the first mouthwash containing fluoride in Vietnam.

AIM: This research was conducted to evaluate the efficacy of HMU Fluorinze mouthwash on remineralizing enamel in laboratory conditions.

METHODS: 20 third molar teeth were cleaned and covered with nail polish, except for a 3 x 3 mm square on their buccal surfaces. These teeth underwent two steps: demineralization using Coca-cola and remineralization for 20 days using standard calcifying solution (control group) and standard calcifying solution + HMU Fluorinze mouthwash 2 times/day (experimental group). The index measured of enamel structure after demineralization and remineralization was assessed by a DIAGNOdent pen 2190.

RESULTS: The indices measured of the control group and experimental group at baseline were 3.65 ± 0.76 and 3.35 ± 0.64 respectively. After demineralization the control group measured 21.78 ± 4.48 and the experimental group, 20.25 ± 2.26 . Following remineralization, the control group scores were 6.30 ± 1.03 and the experimental group, 3.90 ± 1.24 , demonstrating statistical significance ($p < 0.01$) between the two groups. After 20 days, the results for the experimental group did not differ from the original results ($p = 0.272$), in contrast with the control group ($p < 0.01$).

CONCLUSION: Results show that HMU Fluorinze mouthwash is better at remineralizing than standard calcifying solution.

Introduction

Historically, fluoride therapy has been used extensively to prevent dental caries. The fluoride ions replace hydroxyl elements leading to more stable enamel crystals [1]. Alternatively, it was observed that low levels (1 ppm or lower) of fluoride in a dissolved liquid can decrease and even inhibit enamel demineralisation [2] as well as increase mineralization

[3]. Fluoride pathways originate from many different products containing fluoride. Patients at high risk of caries are frequently advised to use a mouthwash and brush-on gel at home, as an adjunct to brushing with a fluoride dentifrice [4]. Rinses containing 100 ppm F or 230 ppm F (0.05% sodium fluoride) are as available over-the-counter and recommended to be used less than three times a day [5].

The re-mineralization effect of fluoride mouthwash on dental enamel and the prevention of

tooth decay have been proven [5], [6], [7]. A meta-analysis study combining the results of 35 trials showed that, on average, there was a reduction of 27% in decayed, missing and filled surfaces on permanent teeth exposed to fluoride mouthwashes compared to those that were not [5]. Another experimental study conducted by Faller RV et al., (2011) demonstrated the ability to prevent tooth decay and remineralization of four fluoride mouthwash products (100 ppm) from ACT, Listerine, and Crest brands [6]. This research showed that treating with any mouthwash had significantly higher concentration of fluoride absorption than using water alone as a rinse. Phan Bhongsatiern et al., (2019) conducted a study comparing the remineralization effect of sodium fluoride (NaF) mouthwash and NaF gel with toothpaste on early tooth lesions. The average amount of mineral absorption from NaF mouthwash and NaF gel is similar, and both greater than that of NaF toothpaste [7].

Most mouthwash brands sold in Vietnam are imported from overseas, with the exception of a few domestic products such as Dr. ECA solution, which has been proved to treat gingivitis [8], and more recently, HMU Fluorinze mouthwash. HMU Fluorinze is a solution developed by Hanoi Medical University that is less expensive than imported products; however, its effectiveness has not yet been demonstrated. Therefore, the aim of this study is to evaluate the effects of HMU Fluorinze mouthwash on enamel remineralization.

Materials and Methods

Twenty human third molar teeth, extracted from people from 18 to 25 years old, due to complications or for orthodontic reasons, were used in this research. The teeth selected for the study were those that were intact with pulpal vitality at the time of extraction. Teeth that were broken, partially cracked or identified as having a loss of enamel or an enamel defect were excluded from the study.

Ethical research

The teeth used in this study were collected with consent from patients. This *in vitro* study was approved by High Technique Center, School of Odonto-Stomatology, Hanoi Medical University.

Preparation

The selected teeth were cleaned with a prophylaxis cup and polishing gel using a slow-speed handpiece, then rinsed under water. The teeth were immersed in a formol solution and stored in the

refrigerator for preservation. The investigators covered the surfaces of each tooth, except for a 3 x 3 mm window on the buccal surfaces, with an acid-resistant layer (nail varnish). The teeth were labelled respectively and stored in saline solution.

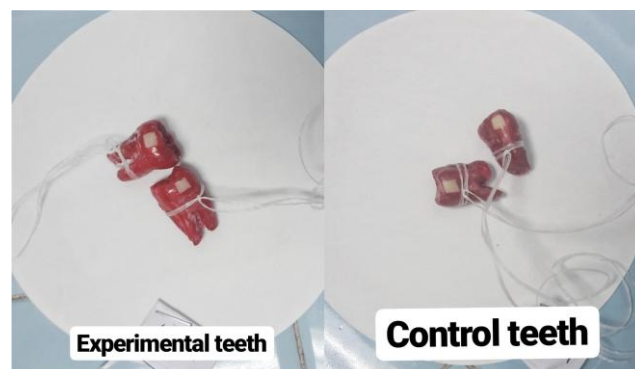


Figure 1: Experimental teeth and control teeth have been labeled

Demineralization

The teeth were immersed in Coca-cola for 20 minutes to demineralize the exposed buccal surfaces of the teeth. Coca-cola the teeth were thoroughly rinsed with water and dried for 1 minute. A DIAGNOdent pen 2190 was used to measure the index before and after demineralization of all teeth.

Remineralization

The four corners of each exposed buccal square were used as reference points. All teeth were randomly divided into two groups. The control group (Group A) was exposed to a standard calcifying solution containing 1,5 mM/L calcium, 0,9 mM/L phosphate and 20 mM/L cacodylate with a pH of 7 for twenty days at 37°C. The calcifying solution (30 ml/tooth) was changed out daily. The experimental group (Group B) received the identical treatment except that a 0.05% NaF solution (HMU Fluorinze mouthwash) was added to the solution twice daily at 8 am and 4 pm, followed by a two-minute rinse with distilled water. At the end of the twenty-day period, the extent of enamel demineralization from the four corners of exposed buccal squares were assessed using the DIAGNOdent pen.

Statistics

The data was analyzed using SPSS 20.0. Pair T-test was used to analyse the difference between demineralization and remineralization stages. The difference between experimental and control groups was investigated using independent T-test. $P \leq 0.05$ was established as statistical significance.

Results

At baseline, the mineralization value of the control group was 3.65 ± 0.76 and that of the experimental group was 3.35 ± 0.64 . There was no significant difference between the two groups ($p = 0.35$) (Table 1).

Table 1: Baseline mineralized value

Group	N	max	min	\bar{x}	SD	p
Experimental group	15	5	2	3.35	0.64	
Control group	15	6	2	3.65	0.76	0.35

After demineralization, the DIAGNOdent pen value of the experimental group (20.55 ± 2.26) and the control group (21.78 ± 4.89) produced similar results ($p = 0.48$) (Table 2).

Table 2: Mineralized value after demineralization

Group	n	\bar{x}	SD	p
Experimental group	15	20.55	2.26	0.48
Control group	15	21.78	4.89	

Differences between these two groups were first identified after remineralization, as shown in Table 3. The index measured for the experimental group and that of the control were 3.90 ± 1.24 and 6.30 ± 1.03 respectively, with a statistical significance of $p < 0.01$.

Table 3: Mineralized value after remineralization

Group	n	\bar{x}	SD	p
Experimental group	15	3.90	1.24	0.000
Control group	15	6.30	1.03	

To compare the measured DIAGNOdent pen values after mineral deposition and post-remineralization, the results in both groups indicated a statistically meaningful difference ($p < 0.01$), shown in Table 4.

Table 4: Compared mineralized value after demineralization to after treatment

	Group	n	Mean	SD	p
	Demineralization-Remineralization	Experimental group	15	16.65	2.59
	Control group	15	15.47	4.96	0.000

However, the value following mineralization in the control group was significantly more compared with the initial mineralized value ($p < 0.01$), whereas the group using HMU Fluorinze mouthwash after 20 days did not differ from the original results ($p = 0.272$) (Table 5).

Table 5: Compared mineralized value after remineralization to the initial period

	Group	n	Mean	SD	p
	Remineralization-Beginning	Experimental group	15	0.55	1.49
	Control group	15	2.65	1.48	0.000

Discussion

In order to create tooth enamel surface lesions similar to dental caries, many authors have used the pH cycle, an experimental method whereby tooth enamel is successively immersed in a demineralizing and then a remineralizing solution, repeating for a certain period of time to form a closed circle. The duration of immersion and the repetitive process between cycles depends on the author and the research purpose [9]. However, the pH cycle requires a solution that has a strict mixing method, which is only available in the laboratory with the appropriate facilities and materials. For the purposes of this study, the mixing portion of the pH cycle was not possible. In this study, the enamel demineralization was performed by Coca-cola immersion ($pH = 2.52$) for 20 minutes [10, 11].

According to Neel et al., 2016, Coke may lead to demineralization in all, the outermost surface, subsurface and superficial demineralisation. Erosion begins when the acids in soft drinks encounter the tooth enamel, which is the outermost protective layer on your teeth. Their effect is to reduce the surface hardness of the enamel. Moreover, they also effect on the surface and subsurface erosion [12], [13].

The teeth for this research were the extracted teeth of people from 18 to 25 years old, the age group that found a percentage no carbonated soft drink consumption of 20.3%, less than once a day 44.7%, once a day 25.4% and two or more times a day 9.6% [14].

In this experimental study, the investigators used the tooth decay evaluation device, the DIAGNOdent pen 2190, which is a reliable tool for detecting the detection and extent of surface lesions [15]. The principle of using a laser beam for diagnosis relies on the fact that an altered mineralized surface irradiated by a longitudinal light wave emits fluorescent radiation. Diagnodent is a diagnostic device that has a probe that emits light directed on the mineralized surface to be examined. If this surface has some form of structural change, it will emit a fluorescent light that is captured back by the probe and the device will display values ranging from 0 to 99 [16], [17].

The results show that the values measured by the DIAGNOdent for the control group were 3.65 ± 0.76 , the research group were 3.35 ± 0.64 , demonstrating that all teeth were without enamel damage. After demineralizing with by Coca-cola for 20 minutes, the DIAGNOdent pen value measured by the control group was 21.78 ± 4.89 and the research group, 20.55 ± 2.26 , indicating all teeth were affected. White spots equivalent to clinical a code of 1-2 according to the International Caries Detection and Assessment System (ICDAS). The difference in means between the two groups was not statistically

significant ($p > 0.05$). After 20 days of remineralization, the DIAGNOdent pen value of the control group was 6.30 ± 1.03 , the experimental group was 3.90 ± 1.24 , all corresponding to that of a sound tooth with a clinical ICDAS code of 0-1. The difference between the experimental and control groups was statistically noticeable ($p < 0.001$). This shows that the mouthwash solution containing Fluoride is more effective at re-mineralizing than the remineralizing solution alone.

Comparing the values of the two groups after post-mineralization with the initial values, the difference in the control group was 18.13 ± 4.48 and the research group was 17.20 ± 2.49 ($p < 0.001$). This result shows the damaging effect of Coca-cola on enamel minerals. Following remineralization, the mineralization value in the control group was 15.48 ± 4.96 and the experimental group, 16.65 ± 2.59 , demonstrating a statistically significance difference. ($p < 0.001$). These measured results of the two groups after remineralization comparing to initial value, this difference in the control group was 2.65 ± 1.48 ($p < 0.001$) and the experimental group is 0.55 ± 1.49 ($p > 0.05$). It suggests that if only using a solution with a mineral composition, the enamel lesions will be recovered but will not reach the initial value before mineral destruction. It is suggested that using mouthwash containing Fluoride may recover mineralization level to baseline.

In 2003, Krithikadatta performed a comparison of the re-mineralization efficiency of three Fluoride-containing products, resulting in NaF mouthwash 0.05% treatment of 18.6 ± 0.9 and after 30 days treatment of 10.47 ± 4.1 [18]. The difference between the two time points demonstrated statistical significance, and showed that NaF mouthwash 0.05% was effective in re-mineralizing tooth enamel. The results of this study are also consistent with the results of the Bahrololoomi et al., study in 2013 investigating toothpaste containing 1450 ppm fluoride using the DIAGNOdent pen 2190 to measure mineralized values [19]. Initial value results were 4.04 ± 2.39 ; after mineral depletion, 11.38 ± 5.95 and following mineral reclamation, 5.48 ± 2.48 .

In conclusion, HMU Fluorinze mouthwash demonstrated enamel remineralization; however, the results were not statistically significant for all groupings and comparisons. Further research is required to confirm the remineralization effectiveness of mouthwashes containing fluoride.

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