

Effect of Remin Pro and Neutral Sodium Fluoride on Enamel Microhardness After Exposure to Acidic Drink

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Abstract

Background and Aim: High consumption of carbonated diet soda is the most common etiologic factor for dental erosion. The purpose of this study was to evaluate the microhardness of the enamel surface eroded by cola after treatment with Remin Pro and neutral sodium fluoride.

Materials and Methods: This in vitro experimental study was performed on 20 extracted premolars with no corrosion, abrasion, crack or hypocalcification. To properly evaluate the microhardness, the buccal surface of samples was polished with sandpaper and then the teeth were immersed in acidic drinks to achieve demineralization. Next, the microhardness was measured at three new points and the samples were randomly divided into two groups for application of neutral sodium fluoride and Ramin Pro. Duration of treatment was in accord with the manufacturer's instructions and the microhardness of the samples was measured again.

Results: Significant differences were found between the two groups after the treatment, and the fluoride group showed higher microhardness ($P=0.04$). Also, the microhardness value was significantly different at the three time points ($P<0.001$).

Conclusion: Cola decreased the enamel microhardness while Remin Pro and neutral sodium fluoride increased the microhardness of enamel. Neutral sodium fluoride showed better remineralizing effect on enamel microhardness.

Key Words: Cola, Tooth Erosion, Hardness, Tooth Remineralization

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Introduction

Tooth is the hardest part of the human body, with a specific structure and constitution. It is composed of three distinct, highly mineralized hard tissues namely the enamel, dentin and cementum. Enamel is the most mineralized part of the tooth and is even harder than iron [1]. Dental enamel has a crystalline lattice structure and is composed of various minerals, the principal component of

which is a complex calcium phosphate mineral called hydroxyapatite [2]. Dental erosion is defined as localized loss of the tooth surface by a chemical process of acidic solutions of nonbacterial origin [3]. Dental erosion is a multifactorial phenomenon that occurs during life, and is largely irreversible. Following erosion of enamel, dentin would be at risk. Dentin compared with enamel is lost more quickly, and there is even the possibility of pulp

damage. The erosive potential of diet depends mainly on its pH, buffering capacity, acidity and consumption patterns [4,5].

Two main patterns are as follows:

1. Exogenous erosion caused by extrinsic factors, e.g. dietary and occupational factors. Usually, the lesion is bilateral, affecting mostly the labial and buccal surfaces of the incisors, canines and premolars adjacent to the gingival margin. Proximal and occlusal surfaces may be involved as well.

2. Endogenous erosion caused by intrinsic factors, e.g. gastric acid. This primarily presents with dissolution of enamel and dentin in the palatal and lingual surfaces of the teeth, with subsequent thinning of the maxillary incisal edges [6].

Soft drinks are an important cause of tooth erosion, because they contain acids such as citric acid, maleic acid and phosphoric acid, which decrease the pH of the oral environment [7]. In addition, soft drinks also contain sugar that plaque microorganisms require for the fermentation process and later produce acids that cause dissolution of the enamel [8]. For the control of dental erosion, several preventive strategies have been proposed and fluoride application is one of them [9]. Usually, the main reason for erosion is exposure to acids. Soft drinks are acidic and have high adhesion to tooth enamel, which leads to development of erosion. Several materials have been introduced for treatment and prevention of erosion, such as fluoride, casein phosphopeptide amorphous calcium phosphate and hydroxyapatite. But a definitive solution has not yet been found. Neutral sodium fluoride is a known material in dentistry and its role in treatment of erosion has been well examined. Fluoride increases the tooth surface hardness and reduces the depth of erosive lesions. Remin Pro is a material which contains xylitol, hydroxyapatite and fluoride. It seems that this substance can prevent demineralization and induce remineralization. Thus, the aim of the present study was to compare the therapeutic action of neutral sodium fluoride and Remin Pro on enamel erosion. The null hypothesis tested was that the effectiveness of Remin Pro on enamel microhardness would be the same as that of neutral sodium fluoride foam.

Materials and Methods

This in vitro, experimental study was conducted on buccal enamel surfaces of 20 sound maxillary first premolars that were extracted for orthodontic treatment or periodontal disease. The teeth were sterilized in 0.2% chloramine T solution for 72 hours at 4°C, and were then stored in distilled water. The surfaces of the teeth were cleaned using fluoride-free prophylactic paste containing pumice and low speed hand piece operating at 500-1500 rpm. Absence of enamel defects, caries and cracks was ensured by evaluation of samples under a stereomicroscope (E24D; Leica, Milan, Italy) at x10 magnification. The crowns were separated from the roots by disks (D&Z, Engelskirchen, Germany) and were mounted in orthodontic resin cylinders. The buccal surfaces were polished using 2500, 3000 and 5000 grit silicon carbide paper. For mounting the teeth, a square-shaped label measuring 3x3 mm was placed on the buccal surface of the teeth. The remaining areas were coated with two layers of acid resistant nail varnish (Maxfactor, Paris, France). In order to avoid the confounding effect of monomer and its polymerization heat, cold mounting polyester resin (Acropars, Tehran, Iran) was used for mounting. The teeth were placed in a container and catalyst was added to the polyester liquid resin in the container. The samples were randomly divided into two groups of 10. Baseline enamel microhardness measurement was done using a Vickers indenter (SCTMC 1000Z, Shanghai, China) which applied 100 g load for 15 seconds. Three indentations were made in different regions of each sample. The distance between the indentations was 20 µ. The microhardness value of each sample was calculated by averaging the value of all three indentations. The pH of cola soft drink and artificial saliva was measured with a pH meter (Senso direct PH110, Lovibond, Berlin, Germany). Each sample was immersed in 33 mL of fresh cola for two minutes at room temperature and then in artificial saliva (pH=7.1). This procedure was repeated three times per day with 8-hour intervals for three days. In order to simulate the clinical situation, the temperature of solutions was adjusted at 9°C. After completion of the first erosion process, samples were rinsed with deionized water and then blotted

dry. For the second time, the enamel microhardness of each sample was measured at three different regions. In one group, a thin layer of Remin Pro (Voco, Cuxhaven, Germany) and in another group neutral sodium fluoride (Pascal, Washington, USA) were applied on the enamel surfaces of the samples by a microbrush, and left undisturbed for five minutes and then stored in artificial saliva for 12-hour intervals for seven days. All samples were stored at 25°C. Then, enamel microhardness of each sample was measured again. The composition of each material is shown in Table 1.

Table 1. Materials used in the present study and their composition

Material	Composition
Cola Soft Drink	Water, sugar, carbon dioxide, caramel color, phosphoric acid, flavor, caffeine
Artificial Saliva	Deionized water, NaCl, CaCl ₂ , NaH ₂ PO ₄ , NaF, NaN ₃
Remin Pro	Hydroxyapatite, xylitol, sodium fluoride
Neutral Sodium Fluoride	0.2% NaF

Results

In this study, 20 samples were analyzed (10 samples in each group). Statistical indices of microhardness are shown in Table 2.

Normal distribution of microhardness data at different time points in each of the two groups was confirmed by the Shapiro-Wilk test ($P=0.094$).

Repeated measures ANOVA showed significant differences between the two groups and fluoride group showed higher microhardness ($P=0.04$); also, the amount of microhardness at the three time points had significant differences ($P<0.001$). The results of pair comparisons using the Bonferroni method showed that the amount of microhardness at baseline and after treatment, were not significantly different ($P=1.0$), while the microhardness after acid exposure was significantly lower than the baseline and the post-treatment values ($P<0.001$ in both cases). The

interaction effect of microhardness measurement condition and group was not significant ($P=0.222$), which means that, as shown in Diagram 1, the decrease and increase in microhardness at different conditions, followed the same pattern in the two groups (i.e., lines are almost parallel).

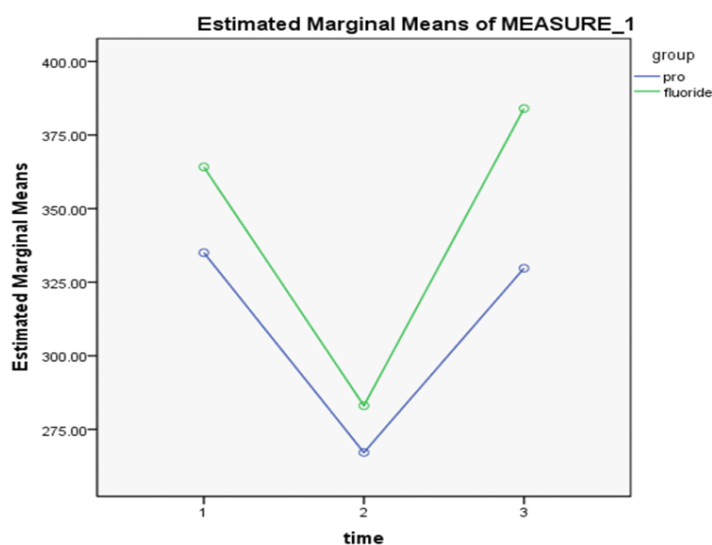
Discussion

Dental erosion is defined as acidic dissolution of tooth surface without involvement of bacteria [10]. Erosion plays a major role in destruction of tooth minerals. Prevalence of erosion has greatly increased in the recent years due to the growing consumption of acidic carbonated soft drinks [11]. Soft drinks have many potential health problems, and can cause dental caries and enamel erosion [12,13]. The methodology of the present study was designed to simulate frequent soft drink consumption in presence of saliva [3]. In the present study, erosive demineralization was induced by the cola drink, which is one of the most widely consumed soft drinks with erosive potential. The erosion process was performed three times (every eight hours) to represent three mealtimes [8]. In this study, an acidic beverage was used freshly for the erosion procedure. Some studies revealed that the beverage should be kept in closed container to avoid the removal of gas that may increase the pH [10,14]. However, the efficacy of Remin Pro and neutral sodium fluoride for reducing tooth erosion has been investigated in several studies [10,14,15]. The aim of this study was to compare the efficacy of Remin Pro and neutral sodium fluoride for treatment of enamel erosion using microhardness test. Various materials have been used for treatment of erosion including casein phosphopeptide amorphous calcium phosphate (MI Paste plus and Tooth Mousse) and fluoride varnish [3,16]. In the present study, Remin pro and neutral sodium fluoride were examined, which are commercially available. Fluoride is very important for remineralization in the oral cavity. The concentration of fluoride in the mouth and in the enamel must be high enough to change the cycle of demineralization to remineralization. With fluoride consumption, fluorohydroxyapatite and fluorapatite crystals are formed in the enamel structure. The critical pH of these crystals is less than that of hydroxyapatite

Table 2. Statistical indices of microhardness[†] at different conditions and groups (n=10)

Group		Mean	Std. Error	95% Confidence Interval for the Mean		Minimum	Maximum
				Lower Bound	Upper Bound		
Baseline Microhardness	Remin Pro	335.0200	16.79525	267.0265	373.0135	245.80	406.80
	Fluoride	364.1400	17.68875	324.1253	404.1547	264.90	466.40
Microhardness after Acid	Remin Pro	267.1500	13.87755	235.7568	298.5432	194.60	326.20
	Fluoride	283.0600	10.53960	259.2178	306.9022	209.90	317.10
Microhardness after Treatment	Remin Pro	329.7500	14.45664	297.0468	362.4532	237.50	413.60
	Fluoride	384.0000	9.75295	361.9373	406.0627	341.90	448.30

[†] The values are expressed in N/mm².

**Diagram 1.** Average microhardness in the two groups at different time points

and equal to 4.5. At a pH of 5.5, when hydroxyapatite crystals begin to dissolve by reducing phosphate and carbonate ions, these

crystals remain stable. Sodium fluoride can be used with other elements such as tin and titanium [17-19].

The effect of fluoride is mainly attributed to deposits such as calcium fluoride on tooth surfaces, which seem to act as a physical barrier, preventing contact between the acid and enamel or as a mineral reservoir, to buffer the acids, and increase remineralization. Formation of a layer of calcium fluoride-like substance and its protective effect on demineralization depend on the pH, the concentration of fluoride, and type of fluoride salt [3]. Several studies have investigated the efficacy of different products such as gels, varnishes, toothpastes and different concentrations of fluoride for treatment of dental erosion [14,20-22]. Remin Pro contains xylitol, hydroxyapatite and fluoride. Research has shown that these materials have a good effect on reducing erosion and promoting remineralization [15,16,22]. In this study, neutral sodium fluoride and Remin Pro were used twice a day (every 12 hours) each time for five minutes for a total of seven days.

In-vitro erosion is greater than in-vivo erosion, because the saliva and its protective effect are not present, even with the use of artificial or collected saliva [3,14]. Artificial saliva is the most important biological factor affecting the progression of dental erosion, which increases the mean enamel hardness after demineralization by cola [14]. One study showed that cola reduced enamel hardness but the hardness was partially restored with artificial saliva. Thus, artificial saliva can reharder the acid-softened enamel [3]. In our study, specimens were stored in artificial saliva, in order to simulate the pH challenges that occur in the oral environment.

Mineral gain or loss in the enamel as a result of demineralization and remineralization can be measured as hardness change [9,16]. Microhardness of the enamel can be measured by Vickers indenters. In the Vickers microhardness test, a diamond in the shape of a square-based pyramid is pressed into the polished surface of a material under a specific load. The test is suitable for determining the microhardness of very brittle materials, such as enamel tooth structure [23]. One factors which may alter the microhardness is the preparation of specimens, because any tilt or irregular surface may yield a too large indentation and thus a smaller Vickers microhardness number. Hence, three indentation areas were made to avoid

any operational bias and then the average of these three indentations was taken for statistical analysis. Therefore, in this study, Vickers microhardness test was used to evaluate the effect of Remin Pro and neutral sodium fluoride on the enamel surface microhardness after consuming acidic beverages, which determines the material resistance to pyramid-shaped indenter [3,8]. The results of this study showed that the microhardness in neutral sodium fluoride group was significantly higher than in Remin Pro group, which may be because the fluoride reduces enamel solubility by participating in the structure of hydroxyapatite, and this reduction depends on the concentration of fluoride [24]. In this study, the microhardness was significantly different in the three stages of the study. Comparison of the stages showed that microhardness after polishing and after treatment was not significantly different, while the microhardness after using the acid was significantly less than that after polishing and after treatment. This means that the acidic drink reduced microhardness. Moreover, treatment with neutral sodium fluoride and Remin Pro increased microhardness up to its baseline level. It means that fluoride in neutral sodium fluoride replaces the hydroxyl ions in the structure of hydroxyapatite and creates fluorapatite. This crystalline structure, in contrast to hydroxyapatite, is more resistant to acid attacks, and is less soluble [17,18]. In Remin Pro, the combination of xylitol, hydroxyapatite and fluoride has a major effect on remineralization. Xylitol interferes with glycolytic pathway, and has an anti-cavity effect that prevents cavity formation. Studies have shown that this material can be used to facilitate the movement of calcium ions in the remineralization process. Hydroxyapatite fills the smallest irregularities in the enamel caused by erosion. It also smoothens the tooth surface. Fluoride is converted to fluorapatite, which is more resistant to acid attacks [15,18]. In this study, neutral sodium fluoride had a greater impact on increasing microhardness compared to Remin Pro; it could be due to eight times higher concentration of fluoride in neutral sodium fluoride than in Remin Pro.

The results showed that acidic beverage reduced microhardness, and treatment with neutral sodium fluoride and Remin Pro led to an increase in

microhardness, but neutral sodium fluoride had a greater impact on increasing the microhardness compared to Remin Pro. In the current study, we tried our best to eliminate all the confounding factors. Further studies are required to investigate any significant interactions between substances. More research is needed for use of these substances in the general population.

Conclusion

Acidic drinks reduce the enamel microhardness but consumption of materials such as Remin Pro and neutral sodium fluoride can remineralize the enamel. Neutral sodium fluoride increased the enamel microhardness more than Remin Pro.

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