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Root Dentin Remineralization by Arginine and Sodium Fluoride

Arginin ve Sodyum Florür ile Kök Dentin Remineralizasyonu

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Abstract

Objective: The aim of this study was to investigate the possible effects of arginine and sodium fluoride on the microhardness of demineralized root dentin.

Materials and Methods: Forty anterior teeth were collected. Disc specimens with 2 mm thickness were prepared. Specimens were divided into 4 groups (10 specimens/group). Microhardness measurements and scanning electron microscopy/energy dispersive X-ray spectrometry analysis were performed before and after the treatment. Statistical analyses were conducted with the software SPSS 19.0.

Results: There was a significant difference between mean values of microhardness ($p<0.05$). There were statistically significant differences among the groups in terms of phosphorus ($p<0.05$) while the difference in calcium was not statistically significant ($p>0.05$).

Conclusion: No effects on microhardness and remineralization were observed by arginine application.

Keywords: Arginine, dental caries, energy dispersive X-ray spectroscopy, fluoride, microhardness, scanning electron microscopy

Öz

Amaç: Bu çalışmanın amacı, arginin ve sodyum florürün demineralize kök dentinin mikrosertliği üzerindeki olası etkisini araştırmaktır.

Gereç ve Yöntemler: Kırk adet anterior diş toplandı. İki mm kalınlığında disk örnekler hazırlandı. Örnekler 4 gruba ayrıldı (10 örnek/grup). Her örneğin mikrosertlik ölçümleri ve taramalı elektron mikroskobu/enerji dağıtıcı X-ışını spektrometresi analizleri, test öncesi ve sonrası yapıldı. İstatistiksel analizler SPSS 19.0 programı ile yapıldı.

Bulgular: Ortalama mikrosertlik değerleri arasında anlamlı fark vardır ($p<0,05$). Gruplar arasında fosfor açısından istatistiksel olarak anlamlı fark bulunurken ($p<0,05$), kalsiyum açısından fark istatistiksel olarak anlamlı değildir ($p>0,05$).

Sonuç: Arjinin uygulamasının mikrosertlik ve remineralizasyon üzerinde herhangi bir etkisi gözlenmemiştir.

Anahtar Kelimeler: Arjinin, diş çürüğü, enerji dağılımlı X-ışını spektroskopisi, florür, mikrosertlik, taramalı elektron mikroskobu

Introduction

Dental caries is still one of the most prevalent and common chronic disease in the world (1). Anti-caries agents and products prevent the development of dental caries by inhibiting the acid production of bacteria or by changing the remineralization balance of the hard tissues of the tooth. In the remineralization process, calcium (Ca) and phosphate are incorporated into the demineralized tooth structure, resulting in a net mineral recovery (2). Many remineralizing agents and remineralizing techniques have been investigated and most are used clinically with positive results (3). Remineralization agents should give Ca and phosphate without causing any side effects or increase the remineralization potential of saliva (4). Fluoride (F) is an effective anti-caries agent. F inhibits enamel and dentin

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demineralization and enhances remineralization. By topical application of high concentration F, before the subsurface of the tooth is remineralized, enamel surface may also be sealed with F. Therefore, F is widely used in studies that examined remineralization (5).

Dentin hypersensitivity due to exposure to dentin in response to thermal, vaporizing, tactile, osmotic, chemical stimuli is the most common problem characterized by short, sharp pain after tooth preparation (6). Altered fluid flow in the dentinal tubules in response to temperature, drying, or osmotic balance fluctuations is the source of the pain. As a result, nociceptor activation occurs in the pulp/dentin border region. The application of sodium F to exposed dentin surfaces allows calcium F crystals to precipitate by saturating the dentin fluid with Ca and phosphate ions, and the canal diameters are narrowed and the impulse transmission stops due to the mechanical occlusion of the dentin canals (7). Osmari et al. (8) concluded that F varnish had an immediate desensitising effect after one single application. Another study showed that sodium F is effective in reducing cervical dentin hypersensitivity (9). All these results support the efficiency of sodium F on dentin hypersensitivity.

Arginine is an amino acid found in saliva and is responsible for raising the pH of saliva (10). Due to its desensitizing effect, in recent years, arginine has been added as an additive to toothpaste and other F-containing dental care products such as mouth rinses, topical F preparations and dental devices (11). Today, protective approaches gained great importance in caries control. Sodium F and arginine are among the agents used in dentin hypersensitivity treatment (12). These applications are economical because they have lower cost and they are easier to use. For this reason, F and arginine are widely used in order to relieve the dentin hypersensitivity, to treat anterior ventilation caries and to provide early remineralization of caries.

The aim of this study was to investigate the potential effects of arginine and sodium F on dentin remineralization by using micro-hardness and scanning electron microscopy (SEM)/energy dispersive X-ray spectroscopy (EDS) analyses of demineralized root dentin. The null hypothesis tested for present study was that application of arginine on root surface did not affect the remineralization efficiency of sodium F.

Materials and Methods

Specimen Preparation

Forty freshly extracted caries free straight single-rooted anterior teeth with similar dimension and morphology were collected from adult patients. Teeth were examined under a stereomicroscope and teeth with root caries, cracks and endodontic treatment were excluded from the study. The selected teeth were cleaned with gauze and a fine brush and soft and/or hard attached tissues were removed with a scaler. After ultrasonically cleaning, teeth were stored in 0.5% thymol solution at 4 °C. The teeth were cut with a slow-speed diamond-saw sectioning machine (Isomet, Buehler, Lake Bluff, IL, USA) under cooling water and disc dentin specimens were removed from cervical one-third of the root. All samples were embedded into acrylic molds and polished with a series of silicon carbide abrasive papers from 600 to 1,200 grit to provide a flat surface. Ethical permission required for the study to be carried out was obtained from the Ethic Committee of Artvin Çoruh University (decision number: 5, date: 16.11.2017).

Preparation of Demineralizing and Remineralizing Solutions

Artificial saliva solution was prepared fresh before the tests according to the same composition described by Ten Cate et al. (13). The solution contains 1,28568 g NaCl, 0,0320 g $MgCl_2.6H_2O$, 0,07945 g $CaCl_2.2H_2O$, 0,29857 g KCl, 0,897 g KOH and 472 µl H_3PO_4.

Remineralization solution was prepared fresh before the tests according to the same composition described by Ten Cate et al. (13) with 1.0 mM $CaCl_2$, 2 mM KH_2PO_4, 150 mM KCl. It was preserved by adding 0.01% NaN_3, pH was adjusted to 7.0 using 1M KOH and kept at room temperature.

The demineralization solution was prepared as a mixture of 100 mmol/L sodium hydroxide and 100 mmol/L lactic acid at pH 5.0. To achieve a viscosity of 100 cp, 0.2 g/L carboxymethyl cellulose sodium salt was added to the solution. The demineralized teeth were treated twice a day in all different mouthwash solutions for one month, as prescribed by each manufacturer.

Dentin disc specimens were immersed in demineralizing solution (DS) for 7 days in capped containers containing 10 mL of demineralization solution for each sample. The DS was changed every 24 h. After artificial incipient caries-like lesion formation, the specimens were removed from DS and thoroughly rinsed with deionized water. For this experimental study, the specimens were randomly divided into 4 groups (10 specimens/group): Group remineralizing solution (RS): control RS, group NaF: 5% NaF, group Arg: 8% Arg, group NaF-Arg: 5% NaF-8% Arg. The samples of all groups were then kept in artificial saliva at 37 °C for 7 days.

Microhardness Testing and SEM/EDS Analysis

The microhardness measurements were performed with a micro-hardness tester (Future Tech FM 800e, Future Tech Corp, Tokyo, Japan) using a Vickers diamond indenter at three different points, 0.5 mm distance from the root canal. The indentations were made equally, perpendicular to the surface of the specimen and each indentation being no closer than 0.5 mm to the other, using a 100 g load with a dwell time of 15 s. The three hardness values for each specimen were averaged and reported as a single value.

SEM (EVO LS 10, Carl Zeiss NTS, Germany), as well as EDS analysis were performed by using a scanning electron

microscope in connection with EDS X-ray detector (EVO LS 10, Carl Zeiss NTS, Germany). SEM images were obtained under 2,000x, 5,000x magnifications, at 20 kV voltage and a working distance of 10 mm. After SEM analysis, the rates of Ca, phosphorus (P), Oxygen (O), and F elements on the root dentin surface were determined in percentages by EDS-X ray detector and Ca/P ratios were calculated.

Statistical Analysis

Statistical analyses were performed with the software SPSS 19.0 (IBM Corporation, Armonk, USA). The Shapiro-Wilk test was used to assess the normality of the distributions. As the micro-hardness measurements before the treatment were not normally distributed the nonparametric Wilcoxon signed-ranked test was used to assess the difference before and after the treatment. Kruskal-Wallis tests were conducted to compare the differences caused by the treatment groups and the Mann-Whitney U test was performed to test the significance of pairwise differences of the treatment groups. One-way ANOVA and pairwise post-hoc tests of Bonferroni and Tamhane's test were used to compare the parameters among the groups for SEM/EDS statistics. A significant level of α=0.05 was set for comparison between the groups.

Results

Microhardness

Table 1 describes the comparison of microhardness values before and after treatment. By Wilcoxon signed-ranked test there is a significant difference between mean values of root dentin microhardness ($p<0.05$). All DSs, except for Group Arg, increased the root dentin microhardness.

Chemical analysis of the demineralized root dentin using EDS showed that it had predominantly O, phosfporus (P), Ca, and F. There were significant differences in O, P, F atomic weight percentages and Ca/P weight percentage (Wt%) ratio except for Ca after the treatment ($p<0.05$). Comparison of the mineral content obtained in each test groups after treatment are shown in Table 2. According to the results of this study, there was statistically significant difference among the groups in terms of P ($p<0.05$) while the difference in Ca atomic percentages was not statistically significant ($p>0.05$). Similarly, there was statistically significant difference between group NaF and group NaF-Arg ($p<0.05$). When compared with group NaF, in group NaF-Arg, the atomic weight percentage of O and P increased while F and Ca/P level decreased.

The SEM micrographs of the remineralized root dentin surfaces for control, NaF and Arg groups in 5,000x magnification are shown in Figures 1-3. Specimens showed some remineralization and crystal structure formation on the surface of the sections. Dentin surface was covered with a homogeneous and dense mineral content and the boundaries of the crystal structure are clearly observed in the SEM micrographs of the control group. Specimens treated with NaF showed a surface layer in which irregular and porous minerals are deposited, while in samples treated with arginine, the surface layer is much more homogeneous and denser than the NaF group.

Discussion

In the present study, the potential effects of arginine and sodium F on root dentin remineralization was investigated. The null hypothesis that application of arginine on root surface did not affect the remineralization efficiency of sodium F, was accepted.

In this *in vitro* study, SEM combination with EDS elemental analysis have been used for the investigation of root dentin surfaces before and after the treatments in the same specimens. Thus, each specimen served as its own control (14). All specimens demonstrated a similar pattern of root dentin surfaces. Several studies have used SEM-

Table 1. Comparison of microhardness values obtained in each test groups before and after treatment using Wilcoxon analysis ($p<0.05$)

	Group 1 (control)	Group 2 (5% NaF)	Group 3 (8% Arg)	Group 4 (5%NaF + 8%Arg)
Before	61.24±3.09	61.82±1.43	59.24±3.77	61.77±1.43
After	86.75±20.56	110.54±21.90	54.87±14.26	64.02±13.10

Table 2. Comparison of the mineral content obtained in each test groups after treatment

n=40	Oxygen	Phospforus	Calcium	Fluorine	Ca/P
Control	67.27±2.49	10.05±0.80	20.08±1.80	2.13±0.86	2.00±0.14
NaF	43.37±6.58*	8.36±1.32*	20.28±1.13	27.64±7.34*	2.49±0.52*
Arg	66.27±2.21	11.05±0.92	19.65±0.80	2.70±0.87	1.79±0.09
NaF + Arg	61.01±6.55*	10.21±1.10*	19.97±1.24	8.52±7.23*	1.97±0.15*

*Statistically significant difference ($p<0.0$)

EDS to evaluate the demineralized tooth surfaces (15-17). The combined use of SEM with EDS elemental analysis is a good tool for investigating demineralization and remineralization. Several studies have been carried out on the tooth demineralization process and the contribution of home oral care products containing specific substances that can remineralize and/or repair the tooth surface in this process (18). It was reported that enamel caries lesions and root caries lesions are quite different because of their morphology and pathogenesis (19). In this study, roots of natural teeth were cut horizontally and the surface of the test specimens was mechanically removed. As a result, root dentin was always at the surface zone. Thus, demineralization process allowed to obtain standardized and comparable root caries lesions.

Microhardness tests are widely used to measure the hardness of teeth. There is no standard condition for enamel and dentin microhardness testing; therefore, selection depended on the researcher's decision. Because of the difference between enamel and dentin microstructure, hardness values depend upon indentation loads or times. In the current study, Vickers microhardness test was used and 100 g load with a dwell time of 15 s was applied. Although the Vickers hardness value for dentin was shown to be between 46 and 53 (20), higher values were obtained in this study. While NaF solution significantly increased the microhardness values, 8% Arg solution significantly decreased the microhardness values as compared to the control group and the baseline. This difference may be due to factors such as sample preparation, diagonal length reading error, variation in chemical composition, age, and location in the tooth.

The remineralizing effect of F was demonstrated by analyzing the microhardness of the enamel surface. In this study, there was no statistically significant difference between the study groups in terms of baseline microhardness values. However, a significant increase in root dentin microhardness was shown after topical application of F in the NaF group in comparison to the baseline values and the control group. It was reported in the literature that a continuous F support is needed to ensure its contribution to mineralization, while it is rapidly removed by saliva (21). Rosin-Grget et al. (22) reported that the samples treated with topical F were different from the control group while globular and crystalline formations occurred on the enamel surface after topical F application. In the present study too, formation of crystalline structures of various shapes and densities were observed in the samples of F group. The crystal structures that cannot be removed from the surface indicate that these formations cannot be taken as ordinary superficial deposits, but are actually adhered to the enamel surface.

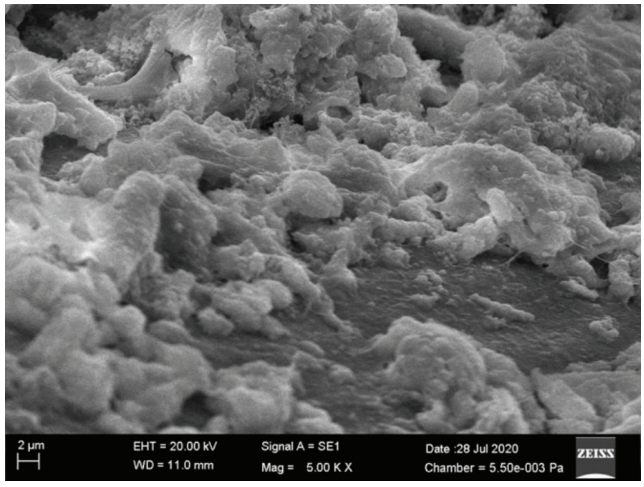

Figure 2. SEM image of NaF group at 5,000x magnification after treatment

SEM: Scanning electron microscopy

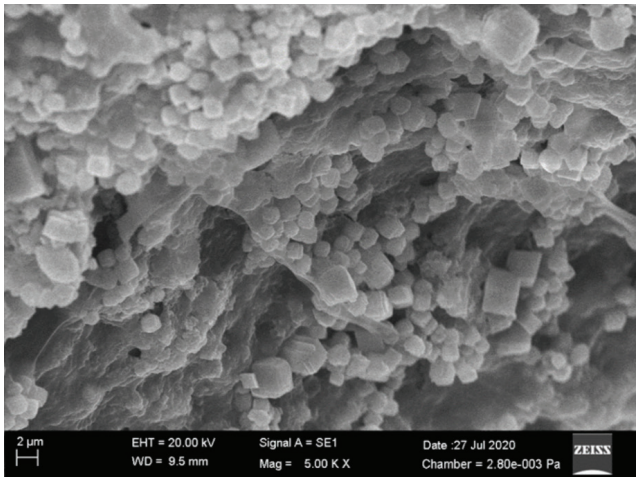

Figure 1. SEM image of control group at 5,000x magnification after treatment

SEM: Scanning electron microscopy

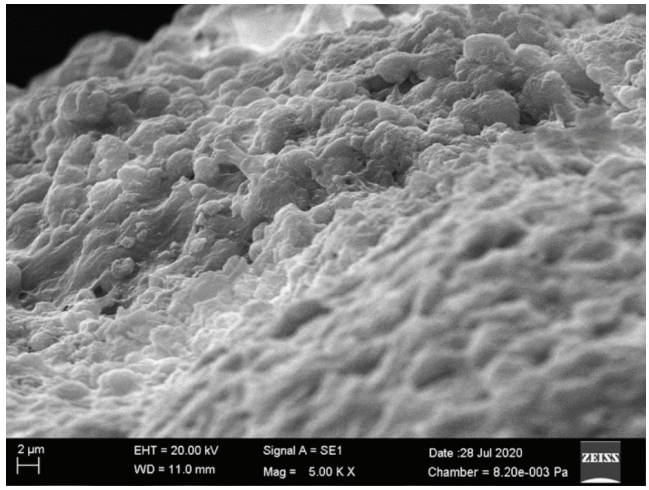

Figure 3. SEM image of Arg group at 5,000x magnification after treatment

SEM: Scanning electron microscopy

Dentifrice products containing arginine/bicarbonate and Ca carbonate, with or without F, have been shown to be significantly effective in reducing dentin sensitivity by plugging and sealing dentinal tubules. Hsua et al. (23) reported that toothpaste containing 8% arginine and Ca carbonate reduced the dentin hypersensitivity. It was demonstrated in clinical studies comparing 8% arginine-containing toothpaste with F-containing toothpastes in the prevention of dentin hypersensitivity, that the sensitivity can be effectively relieved after 8 weeks and natural protective benefits can be achieved for oral care (24). Unlike the literature, 8% arginine application decreased the microhardness level in this study. We could not obtain compatible results with the literature. However, the microhardness level decreased in the NaF + Arg group in comparison to both baseline values and control group. The fact that such a result obtained in the NaF + Arg group suggested the question that arginine might have inhibited the effect of F and eventually reduced its effectivity. In addition, the effect of 8% arginine application on remineralization was not statistically significant different in comparison to the control group. In the present study, it was not observed any positive effects of 8% arginine application on both microhardness and remineralization. Moreover, SEM results of this group were found to be more homogeneous and more intense than the other groups.

There are some limitations of this *in vitro* study. The origin of the human teeth that were used for this experimental study was unknown. Because, age, sex, date of the extraction and how long they were stored in solution was not recorded. This may explain the significant differences of this study results when compared with the previous similar studies.

Conclusion

Although there is a wide range of products available in the treatment of dentin hypersensitivity, further studies should be conducted to develop more effective products or to increase the effectiveness of existing products. Within the limitations of this study strengthening of dentin surfaces was provided by 5% NaF while use of arginine did not make any contribution.

Ethics

Ethics Committee Approval: Ethical permission required for the study to be carried out was obtained from the Ethic Committee of Artvin Çoruh University (decision number: 5, date: 16.11.2017).

Informed Consent: Informed consent is not required.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: S.S.B., Concept: S.S.B., F.B., Design: S.S.B., F.B., Data Collection or Processing: S.S.B., Analysis or Interpretation: S.S.B., E.D., Literature Search: S.S.B., E.D., Writing: S.S.B., E.D.

Conflict of Interest: No conflict of interest was declared by the authors.

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