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Clinical assessment of demineralization and remineralization surrounding orthodontic brackets with FluoreCam

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ABSTRACT

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Keywords: Demineralization Remineralization FluoreCam Orthodontic brackets MI Paste Plus Remin Pro **Objective:** To determine quantitatively the amount of demineralization and the ability of commercially available products and an experimental cream to inhibit or reverse ortho-dontic related demineralization.

Methods: A total of 20 patients who were 25-35 years old and having orthodontic treatment for 6-8 months were chosen. Caries risk assessments were done for each patient and ones with "moderate risk" were included. Patients with fixed orthodontic appliances were divided into 4 groups (5 patients each) including one control and 3 study groups. All patients used same toothpaste 2 times a day during the 3 weeks study period. Additional to the toothpaste first study group used MI Paste Plus (GC, Tokyo, Japan), second study group used Remin Pro (Voco, Cuxhaven, Germany) and third group used an experimental remineralizing cream per day for 3 weeks. Maxillary central and lateral incisors of each patient were examined by FluoreCam (Daraza Therametric Technologies, USA) device. The examinations were performed at baseline and at the end of 1st, 2nd and 3rd weeks. **Results:** According to the FluoreCam measurements the control group showed significant amount of demineralization at the end of 3 weeks, moreover the amount of demineralization has gradually increased in time. At the end of the study all 3 study groups showed significant amount of remineralization and the amount of remineralization for all the 3 study groups has gradually increased in time. However the amount of remineralization for 3rd study group was lesser than the 1st and 2nd study groups. The remineralization amounts for the 1st and 2nd study groups were determined to be identical.

Conclusions: This study demonstrated that demineralization is measurable around orthodontic brackets and the demineralization can be completely inhibited and/or reversed by the use of commercially available remineralization products.

1. Introduction

White spot lesions are clinically detectable demineralization areas defined as opaque, white areas caused by the loss of minerals below the outermost enamel layer. These demineralization centers may cause a significant clinical problem when surrounding orthodontic brackets during orthodontic treatment [1–3]. The prevalence of white spot lesions has been reported between 2% and 96% in orthodontic patients [4,5].

Approximately 50% of orthodontic patients develop white spot lesions in at least 1 tooth however this ratio is only 24% in those not undergoing orthodontic treatment [3]. Clinically detectable white spot areas can be visible even 1 month after the placement of fixed orthodontic appliances [3,4]. The irregular surface of the orthodontic brackets, wires, bands and other attachments potentially causing retentive areas for plaque and with the contribution of poor oral hygiene, dietary carbohydrate and salivamodified bacterial infection, early demineralization in dental enamel may occur [4–8]. Lesions may remain as cavity-free demineralization areas as well as caries lesions with cavities in long-term [9].

The ideal method for assessing the early caries lesion should be valid, simple, reproducible and also non-invasive [3]. Optical methods are one of the best options in order to achieve those necessities. Light-induced fluorescence (QLF), electrical caries monitor, digital imaging fiber-optic trans illumination and

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another auto-fluorescence based device were used; FluoreCam may be defined as the most common optical diagnostic methods in today's dentistry [1]. Fluorescence can be defined as a result of light absorption and the principle of QLF is the detection of auto-fluorescence from dental tissues [10]. As the demineralization in enamel occurs, minerals are replaced causing less light absorption by the enamel tissue. So the intensity of fluorescence decreases in demineralized areas appearing in darker color compared to sound dental areas [11,12]. Light-induced fluorescence has been used in dentistry for detection and quantification of demineralization areas and early caries lesions. QLF device was also used in many clinical studies and validated in most of them [13-15]. The detection of demineralization areas is possible before they are visible by using light-induced fluorescence device providing a chance for more conservative, preventive and regenerative treatment options in order to minimize the potential damage [16]. The detection device used in this study FluoreCam is also based on detecting auto-fluorescence from dental tissues like QLF.

Researchers have used several biomaterials on teeth of orthodontic patients in order to prevent demineralization and also enhance remineralization especially around orthodontic brackets [3]. Patient related factors such as diet, oral hygiene, salivary flow as well as dentist related factors such as content of adhesive material used for bracket bonding, content of the toothpaste, additional fluoride varnishes, mouthwashes and pastes may affect the decalcification and regeneration potential of enamel around orthodontic brackets. So dentists have tried to find the most suitable material and method for bonding orthodontic brackets for many years [1-3,17-20]. Fluoride ion is believed to be very effective for preventing dental caries through several mechanisms such as: reduction of acid production of bacteria, inhibition of intracellular and extracellular enzymes and replacement of hydroxide ions with fluoride ions in hydroxyapatite thus creating acid-resistant fluorapatite crystals [21,22]. Taking this into account some commercially available fluoride-containing pastes additional to the toothpastes may be used for increasing the regenerative capacity of enamel such as: MI Paste and MI Paste Plus (GC, Tokyo, Japan), Tooth Mousse (GC, Tokyo, Japan), Remin Pro (Voco, Cuxhaven, Germany), Duraflor (Medicom, Canada), Pro Varnish (Premier, USA).

The purpose of this *in vivo* study was to determine the amount of orthodontic related demineralization surrounding brackets quantitatively by using FluoreCam, also to assess the ability of some commercially available products and an experimental cream to inhibit or reverse the demineralization.

2. Materials and methods

2.1. Selection of the patients and the teeth

A total of 20 patients aged 25–35 years old and having orthodontic treatment for 6–8 months were selected. The patients had fixed orthodontic appliances and white spot lesions on labial surfaces of maxillary anterior incisors surrounding the brackets. The international caries detection and assessment system (ICDAS) was used for the clinical examinations and the labial surfaces of the maxillary incisors were detected and the ones having scores of '1' and '2' pointing 'early stage decay' were included into the study. ICDAS Score 1 demonstrates visual change in enamel as white spot lesion only with air blasting, while ICDAS Score 2 with and without air blasting (Table 1).

Table 1	
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ICDAS detection	ICDAS layer terms	ICDAS dental terms
6 5 4 3 2 1 0	Established decay Early stage decay	Extensive cavity with visible dentin Distinct cavity with visible dentin Underlying dentin shadow Localized enamel breakdown Distinct visual change in enamel First visual change in enamel Sound surface

2.2. Study design

The patients were divided into 4 groups including 5 patients each - one control group and three study groups. All the patients used the same toothpaste, Ipana Pro-Expert Clinic Line Enamel Regeneration (Crest, OH, USA) two times a day during the three weeks study time. The toothpaste used in this study contains 1100 ppm fluoride, stabilized stannoz and polyphosphate and having less abrasive particles for regeneration of demineralized enamel areas. The control group used only this toothpaste for three weeks. Additional to the toothpaste the patients in first study group used MI Paste Plus (GC, Tokyo, Japan) per day. Stimulating saliva production and restoring minerals are the main features of this enamel regeneration product. It contains casein phosphopeptide-amorphous calcium phosphate, which works for enhancing to remineralize dental tissues [2,3]. The patients in the second study group used Remin Pro (Voco, Cuxhaven, Germany) per day additional to the toothpaste. It is neutralizer of harmful dental plaque acids so that prevents the decalcification of dental tissues. It contains fluoride, hydroxyapatite, and xylitol that reinforce remineralization and strengthen the enamel tissue [22]. The patients in the third group used an experimental remineralizing cream per day additional to the toothpaste for three weeks period. This experimental cream contains honey and ginger that are found effective on remineralization of initial enamel lesion with 50 µm depth [23,24].

2.3. Assessment of mineral content - FluoreCam

Labial surfaces of maxillary central and lateral incisors of each patient were examined by using FluoreCam device (Daraza Therametric Technologies, USA) based on an innovative approach to the quantification of enamel health [25]. The FluoreCam system is also called as fluorescence enamel imaging. As the dental enamel is a highly mineralized tissue, certain light wavelengths exposure may lead it to fluorescence. Additionally the semi-translucent structure of enamel results in different levels of fluorescence emits in different densities. As a result, FluoreCam technology can measure the density of dental enamel by measuring the fluorescence inside [25]. FluoreCam consists of an intraoral camera connected to a specialized computer software and can be applied easily in dental clinic. Demineralization areas in enamel tissue can be seen as marked simultaneously on computer screen. The software has also the ability to record patient's detailed data and readings. In this study the FluoreCam examinations were performed at the baseline and at the end of the 1st, 2nd and 3rd week by two different observers. Measurements were done by holding the intraoral camera directly to the tooth surface from ~1 cm distance. The digital picture of the tooth surface appeared on the computer screen and the areas with less mineral content

surrounding orthodontic brackets were signed automatically by the system software.

2.4. Statistical analysis

The collected data were saved in files of each patient as visual and also quantitative. The quantitative data was exported in every measurement as 'size (the area of lesser mineral content)', 'intensity (the amount of mineral loss)' and 'impact (the product of size multiplied by intensity)' scores and the size and intensity scores were taken into consideration and recorded. The differences between the groups for each measurement period were calculated by using paired samples test and student *t*-test. The mean values of the data for each measurement period of each group were used.

3. Results

According to the results, the scores of all groups were different at all the evaluation periods including the baselines. The baseline and the weekly size and intensity scores between the groups were statistically significant (P < 0.01). The increase in the size scores and decrease in the intensity scores of the control group (toothpaste group) were statistically significant in each measurement period (P < 0.01). On the contrary the decrease in the size scores and the increase in the intensity scores for all study groups were statistically significant in each measurement period (P < 0.01) (Tables 2 and 3).

Tokyo, Japan) and 1100 ppm toothpaste (Colgate, New York, USA) around the brackets for remineralization clinically [1]. The QLF was reported to assess the demineralization and remineralization around orthodontic brackets and as a result demineralization was reported as measured by using any of the methods used [1]. In an in vitro study two different fluoridecontaining varnishes, Duraflor (5% NaF) and Pro Varnish (5% NaF and ACP) were used around orthodontic brackets. Assessments were made with microhardness test and both varnishes were determined to have similar demineralization inhibitor effect [17]. Banks and Richmond used a viscous chemically-cured sealant and bonding system (Maximum Cure), a non-viscous visible light-cured sealant and bonding agent (Transbond, 3M, Japan) for bonding orthodontic brackets clinically and assessed by using a modified index by direct clinical observation. They reported that 75% of the patients were affected by decalcification [18]. In another study fluoride-releasing glass-ionomer cement was used for bonding orthodontic brackets clinically and assessments were made by using 'Taves Diffusion Method'. The caries lesions were reported as prevented [19]. Schmit et al. used a fluoride-releasing cavity varnish, a resin modified glass-ionomer cement and a composite resin for bonding brackets on extracted teeth. They used with polarized light microscopy for assessments and reported that although fluoride varnish couldn't stop demineralization, it helped to reduce lesion formation [20]. Robertson et al. used MI Paste Plus (GC, Tokyo, Japan) to prevent demineralization in orthodontic patients. They assessed the number of white spot

Table 2

The probability comparison of the FluoreCam size scores of the groups.

FluoreCam (size scores)	Control group (toothpaste)	1st Group (MI Paste Plus)	2nd Group (Remin Pro)	3rd Group (exp. cream)	P^{a}
Baseline	2.11 ± 0.93	3.22 ± 1.29	4.43 ± 1.41	3.83 ± 1.11	0.01*
1st week	3.80 ± 0.93	2.99 ± 0.99	4.01 ± 1.83	3.75 ± 0.77	0.01^{*}
2nd week	4.69 ± 0.82	2.16 ± 0.90	3.67 ± 0.55	3.52 ± 1.93	0.01^{*}
3rd week	6.46 ± 0.74	1.31 ± 1.06	2.93 ± 1.71	3.35 ± 0.44	0.01^{*}
Baseline-1st week (P^b)	0.001**	0.001^{**}	0.001^{**}	0.001^{**}	
1st week-2nd week (P^b)	0.001**	0.001**	0.001^{**}	0.001^{**}	
2nd week-3rd week (P^b)	0.001**	0.001**	0.001**	0.001^{**}	

Values are expressed as mean \pm SD.^a: Student *t*-test; ^b: Paired samples test. ^{*}: P < 0.01; ^{**}: P < 0.001.

Table 3

The probability comparison of the FluoreCam intensity scores of the groups.

FluoreCam (intensity scores)	Control Group (toothpaste)	1st Group (MI Paste Plus)	2nd Group (Remin Pro)	3rd Group (exp. cream)	P^{a}
Baseline	-8.96 ± 2.04	-12.13 ± 3.92	-32.13 ± 5.62	-28.63 ± 2.87	0.01*
1st week	-14.11 ± 5.29	-11.61 ± 6.19	-30.33 ± 7.83	-28.43 ± 3.53	0.01^{*}
2nd week	-16.59 ± 4.08	-10.73 ± 3.65	-29.78 ± 2.81	-28.12 ± 0.11	0.01^{*}
3rd week	-21.42 ± 3.69	-9.32 ± 3.26	-27.57 ± 9.01	-27.98 ± 4.41	0.01^{*}
Baseline-1st week (P^b)	0.001^{**}	0.001**	0.001^{**}	0.001^{**}	
1st week-2nd week (P^b)	0.001**	0.001***	0.001**	0.001**	
2nd week-3rd week (P ^b)	0.001**	0.001**	0.001^{**}	0.001^{**}	

Values are expressed as mean \pm SD.^a: Student *t*-test; ^b: Paired samples test. ^{*}: P < 0.01; ^{**}: P < 0.001.

4. Discussion

Researchers have been trying to find more suitable materials and methods to solve the significant clinical problem of fixed orthodontic treatment, the enamel demineralization and mainly the white spot lesions. Ultrasonic system was used for detecting early caries lesions and reported as a promising diagnostic tool [9,26]. Sudjalim *et al.* used a glass-ionomer cement (Fuji Ortho LC, GC, Tokyo, Japan) and a composite resin (Transbond XT, 3M, Japan) in orthodontic bands and applied Tooth Mouse (GC, lesions per surface by using photographic method and an enamel decalcification index. The number and incidence of the white spot lesions were reported as decreased [3]. In another study, MI Paste Plus (GC, Tokyo, Japan) and Prevident Fluoride Varnish (Colgate, New York, USA) were used for treating white spot lesions during orthodontic treatment and digital photographs were used for the assessments. According to the results both materials didn't appear to be more effective than routine home care [2]. The effects of fluoride varnish, casein phosphopeptide-amorphous calcium phosphate complex (CPP- ACP) and Remin Pro (Voco, Germany) on demineralized enamel surface were respectively assessed and according to the microhardness results, CPP-ACP had higher positive effect compared to the other regenerative agents [22]. Gocmen *et al.* assessed the effectiveness of FluoreCam device in monitoring molar-incisor hypo-mineralization and reported it as a promising device in monitoring hypo-mineralization, also providing visual and quantitative feedback to patients [24].

In the present *in vivo* study, a commercially available optical diagnostic method, FluoreCam, was used for detecting demineralization and remineralization areas surrounding orthodontic brackets. The specifications such as clinically validity, simplicity, reproducibility and non-invasiveness are the main reasons for choosing it for the study.

The Ipana Pro-Expert Clinic Line Enamel Regeneration toothpaste used in our study is a commercially available product and also accepted as effective on enamel regeneration. It is well recognized that less abrasive and conservative toothpastes are better choices for the patients with high caries risk such as the ones using fixed orthodontic appliances [27]. According to this the toothpaste chosen for our study would be a correct choice for the accompanying patients.

MI Paste Plus and Remin Pro were also commercially available supplementary used enamel regenerative agents in different studies. Those pastes were reported as effective materials on reversing early enamel demineralization on reported studies [2,3,22].

As the biocompatibility is a must for a newly developed medical product, natural products are more popular in today's dentistry. Researchers have been trying to develop better dental products containing almost 100% natural ingredients [28]. Various herbs using in alternative medicines like honey, ginger, onion, clove, garlic, cinnamon and lime have been tried for their antibacterial effect and mostly found to be successful [29-31]. Accordingly the experimental enamel regeneration cream, used in this study contains ginger and honey. Honey is a mixture of sugars mainly fructose and glucose. It is only recently understood that honey has antiseptic and antibacterial properties as containing hydrogen peroxide (H₂O₂), methylglyoxal and bee defensin-1 [32,33]. Honey was used as a transporter of ginger in our study. Premkishore et al. used ginger with honey against Streptococcus mutants in an in vitro study, assessed by using antibiotic sensitivity test and reported considerable antibacterial activity against S. mutants [28]. Especially ginger is known as a strong antioxidant and an effective agent in reducing inflammation, inhibiting prostaglandin biosynthesis and suppressing immune system's production of cytokines and chemokines without any side effects. These effects make it comparable to non-steroidal, anti-inflammatory medicines. From dental terms it is antibacterial [34], antifungal [35], saliva enhancer [36], toothache painkiller and have the ability to stimulate the apatite that is making this herbal a potential natural regenerative agent for the treatment of enamel demineralization [36]. As supporting views, Weli and Mohammed used ginger extracts to inhibit the S. mutants and reported that the S. mutants concentration showed highly significant reduction in the group used the experimental ginger extract [34].

Upon analyzing the results, statistically significant differences in baseline size and intensity scores between the groups means the control and the study groups were not homogenous. Although the selected teeth of the patients in both control and study groups were considered as early caries lesions by using the criteria of method, ICDAS, the FluoreCam system as a more sensitive method ^[25] could detect the mineralization differences of the teeth in different groups. This heterogeneity can be explained with this sensitivity of the diagnostic method used in the study. As the baseline size and intensity scores were statistically different, statistically different weekly measurement scores between the groups were expected.

The statistically significant increases in size scores of the control group (toothpaste group) at the end of each weekly measurement can be interpreted as the area of lesser mineral content gradually increased around orthodontic brackets. Statistically significant decrease in intensity scores of the control group at the end of each weekly measurement may indicate that the amount of mineral loss gradually increased around the brackets. These results in common may demonstrate that the enamel surrounding orthodontic brackets gradually demineralized in control group and FluoreCam device could detect the weekly mineral content alterations.

Statistically significant decreases in size scores of 1st (MI Paste Plus), 2nd (Remin Pro) and 3rd (experimental cream) groups at the end of each weekly measurement may indicate the area of lesser mineral content decreased gradually around orthodontic brackets. Statistically significant increase in intensity scores of 1st, 2nd and 3rd study groups in time can be interpreted as the amount of mineral loss decreased gradually around the brackets. These results in common may demonstrate that the enamel surrounding orthodontic brackets gradually remineralized in all study groups but the control group and the FluoreCam device could detect the weekly mineral content alterations.

Moreover in three weeks period, less decrease in size and less increase in intensity scores were recorded for 3rd (experimental cream) group compared to 1st (MI Paste Plus) and 2nd (Remin Pro) groups. The experimental cream was an effective agent on preventing and also reversing the demineralization, even though 'MI Paste Plus' and 'Remin Pro' agents were more effective, under the conditions of this study. When comparing 1st and 2nd study group scores, there was no statistically significant difference between 'MI Paste Plus' and 'Remin Pro' in three weeks period indicating that these two regenerative agents had identical effects on preventing and also reversing the demineralization under the conditions of this study. Both 'MI Paste Plus' and 'Remin Pro' agents contain high amount of fluoride almost comparable to fluoride toothpaste. Additionally CPP-ACP and hydroxyapatite in order would have double enhancing effect on remineralization. However high amount of fluoride content may increase the fluoride toxicity risk in longterm usage of these products. On the other side, the mixture's content is completely natural products and safe on fluoride toxicity risk since no additive fluoride. This natural mixture was found more effective than control group in the results obtained. Alternatively may be the FluoreCam device used in this study was not sensitive enough or the study period was not long enough to measure the possible different scores of 2nd and 3rd study groups. Although Durmus et al. reported FluoreCam as a promising method in monitoring hypo-mineralization [25], further studies including comparative diagnostic methods are needed for more precise results.

The groups of the study were heterogenous at the baseline and weekly measurements according to the both size and intensity scores. The FluoreCam device used was a more sensitive diagnostic method than visual inspection in detecting enamel demineralization under the conditions of this study. The amount of mineral loss gradually increased and the demineralization areas expanded in the teeth of the toothpaste group in each weekly measurement. The amount of mineral loss gradually decreased and the demineralization areas shrinked in the teeth of the MI Paste Plus group, the Remin Pro group and the experimental cream group in each weekly measurement. This study demonstrated that demineralization and remineralization are measurable around orthodontic brackets and the demineralization can be completely inhibited and/or reversed by the use of these products. The effects on preventing or reversing the demineralization of 'MI Paste Plus' and 'Remin Pro' agents were determined as equivalent under the conditions of this study. The experimental cream was determined as an effective agent on preventing and also reversing the demineralization under the conditions of this study. Even though the known uses are minimal and further research on this experimental herbal-based cream is required to know its potential benefits in dentistry, it is thought to have an effective role on preventing early enamel demineralization and also on reversing it. This study also demonstrated that orthodontic brackets are potential demineralization causes on surrounding enamel tissue. Patients with orthodontic brackets should use additional remineralizing agents besides fluoridated toothpastes to reverse demineralization effects and to enhance remineralization (P < 0.01).

Conflict of interest statement

We declare that we have no conflict of interest.

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