Evaluation of The Effects of Different Remineralization agents on Initial Enamel Lesions by Scanning Electron Microscope and Energy-Distributed X-ray Analysis

munevver kilic[1] and Taskin Gürbüz[2]

[1]Ataturk Universitesi
[2]Istanbul Medeniyet University

February 1, 2021

Abstract

Aim: The present study compares the effectiveness of four different remineralization agents on the demineralized enamel of permanent human incisors. The purpose of this study is to evaluate the effectiveness of remineralization agents on the initial enamel lesion. Material- Methods: Crowns affected by demineralization were divided into two equal parts. The right halves of the teeth were subjected to no other processes after demineralization for control purposes and were kept in artificial saliva for the duration of the experiment. The left halves of the crowns were remineralized using a 5% NaF-containing fluoride varnish (GC MI Varnish GC Corp, Tokyo, Japan), a casein phosphopeptide and amorphous calcium toothpaste (GC Tooth Mousse, GC Corp, Tokyo, Japan), fluoride, hydroxyapatite, and xylitol, containing a water-soluble remineralization paste (Remin Pro, Voco, Germany) and calcium, magnesium and phosphate-containing gel (Medical Remineralizing gel (R.O.C.S. Trading GmbH, Munich, Germany). After a 21-day remineralization process, and SEM/EDX analysis was performed, and a One Way Anova was used for statistical analysis. Results: According to EDX analysis, the R.O.C.S. medical remineralizing gel was found to have a lower Ca/P ratio than the other material groups (p =0.04). Remin Pro, Voco, and the R.O.C.S. were higher than the control group in evaluating the Ca / P ratio (p=0.014), (p=0.025). Conclusions: The R.O.C.S group, treated with fluoride-free xylitol-containing, a remineralization agent, showed the lowest Ca/P ratio. Compared to demineralized halves of the teeth (control groups), Remin Pro, Voco, and R.O.C.S. medical remineralizing gel may be considered as affecting on initial enamel lesions. Key Words: Remineralizing Agents, EDX, SEM, Enamel,

Aims of the study: The present study compares the effectiveness of four different remineralization agents on the demineralized enamel of permanent human incisors. The purpose of this study is to evaluate the effectiveness of remineralization agents on the initial enamel lesion.

Methods used to conduct the study: Crowns affected by demineralization were divided into two equal parts. The right halves of the teeth were subjected to no other processes after demineralization for control purposes and were kept in artificial saliva for the duration of the experiment. The left halves of the crowns were remineralized using a 5% NaF-containing fluoride varnish (GC MI Varnish GC Corp, Tokyo, Japan), a casein phosphopeptide and amorphous calcium toothpaste (GC Tooth Mousse, GC Corp, Tokyo, Japan), fluoride, hydroxyapatite, and xylitol, containing a water-soluble remineralization paste (Remin Pro, Voco, Germany) and calcium, magnesium and phosphate-containing gel (Medical Remineralizing gel (R.O.C.S. Trading GmbH, Munich, Germany). After a 21-day remineralization process, and SEM/EDX analysis was performed, and a One Way Anova was used for statistical analysis.

Results of the study: According to EDX analysis, the R.O.C.S. medical remineralizing gel was found to have a lower Ca/P ratio than the other material groups (p =0.04). Remin Pro, Voco, and the R.O.C.S. were higher than the control group in evaluating the Ca / P ratio (p=0.014), (p=0.025).

Conclusions drawn from the study and clinical implications: The R.O.C.S group, treated with fluoride-free xylitol-containing, a remineralization agent, showed the lowest Ca/P ratio. Compared to demineralized halves of the teeth (control groups), Remin Pro, Voco, and R.O.C.S. medical remineralizing gel may be considered as affecting on initial enamel lesions.

Key Words: Remineralizing Agents, EDX, SEM, Enamel**,**

What's Known:

1-

It is known in the literature that initial caries is treated. Fluoride and casein are widely used in their treatment, and it is being investigated which other medicaments will work in this treatment.

What's New:

The preset study puts forward an experimental procedure for the evaluation of the success of remineralization agents used for the treatment of initial enamel lesions. The most significant advantage of this procedure is the comparison of demineralized tooth halves and remineralized tooth halves obtained from the same tooth. Thus, we think that the contradiction different enamel compositions in other humans between the control group and the experimental groups has disappeared.

There have been very few studies to date investigating such remineralization agents as Remin Pro and ROCS® Medical Mineral Gel. In the present study, these two materials, for which there is little available information, were evaluated.

1 Introduction

Today, the progression of initial enamel lesions has been significantly reduced by early diagnosis and treatment, with attempts to protect the tooth's enamel and dentin tissue as much as possible.[1]The hard tissues of the teeth are affected by pH values that change in the oral environment. When this value falls below 5.5, the calcium and phosphate ions in the enamel structure migrate away from the tooth tissue, and a destructive process called "demineralization" begins. If some factors do not control this critical pH value, the tooth's destruction continues, and the enamel tissue softens and becomes porous.[2] In the long term, cavitations begin to appear on the surface. Initial enamel lesions are the earliest stage of decay, and at this stage, the caries is limited in the enamel and can prevent from progressing with appropriate treatments.[3,4]However, when the demineralization cannot stop, it moves on from the enamel surface to the dentin layer. Remineralization in these regions is not possible after the cavitation.[5] Between demineralization and remineralization processes, there is a balance. If demineralization increases, caries progresses.[3] If remineralization increases, caries may heal. Prophylactic applications may prevent demineralization from occurring.[3,4] It can also remineralize newly formed demineralized fields.[6]Preventive treatments that encourage the remineralization of initial enamel lesions include improved oral hygiene, arranged eating habits, the regular application of fluoride gels and varnish seans, and the consumption of products with casein phosphopeptide content.[7]

Energy-distributed X-ray analysis (E.D.X.) is a microanalytic technique used to predict mineral quantities in a particular tooth sample. That works by measuring X-rays emitted against their energies. The energy spectrum is obtained against the relative numbers of the detected X-ray and evaluated for the qualitative and quantitative detection of the sample elements using a computer-based program. Moreover, a scanning electron microscope (S.E.M.) is a microanalytic technique used to samples structural analyses.[8]

This in vitro study compares the results of an SEM/EDX analysis following the remineralization of human anterior maxillary tooth enamel using four different materials: GC MI Paste plus; G.C. Tooth Mousse; Remin Pro Voco; and a R.O.C.S. medical remineralizing gel.

2 Material and Method

2.1 Determining the Sample Size

To determine the sample size, effectiveness sizes are calculated as 1.5: alpha error = 0.05 and beta error = 0.20 based on Rallan et al.[7] accordingly, six samples were found sufficient for each group, although considering the potential data loss, decided eight samples for each group (n=8).

2.1 Selection and Preparation of Teeth

A total of 40 primary anterior maxillary teeth with no caries, hypoplasia, or defects were collected over one month. The surfaces of the teeth were cleaned with a polishing brush and tissue residue removed. The teeth were kept in a 0.5% chloramine T solution for 24 h for sterilization before demineralization and then retained in distilled water at room temperature for one week. Before processing, the teeth' crowns and roots were separated from each other with a transverse section with a diamond disc help. Then crowns were then embedded in acrylic blocks with the buccal surfaces of the crowns left open. The crowns set in acrylic were then cut from the inside-gingival aspect and divided into two equal parts (Figure-1). A 3mm2 area was left open on the buccal surfaces of parts A and B, and the remaining areas were closed with nail polish to avoid acid impact.

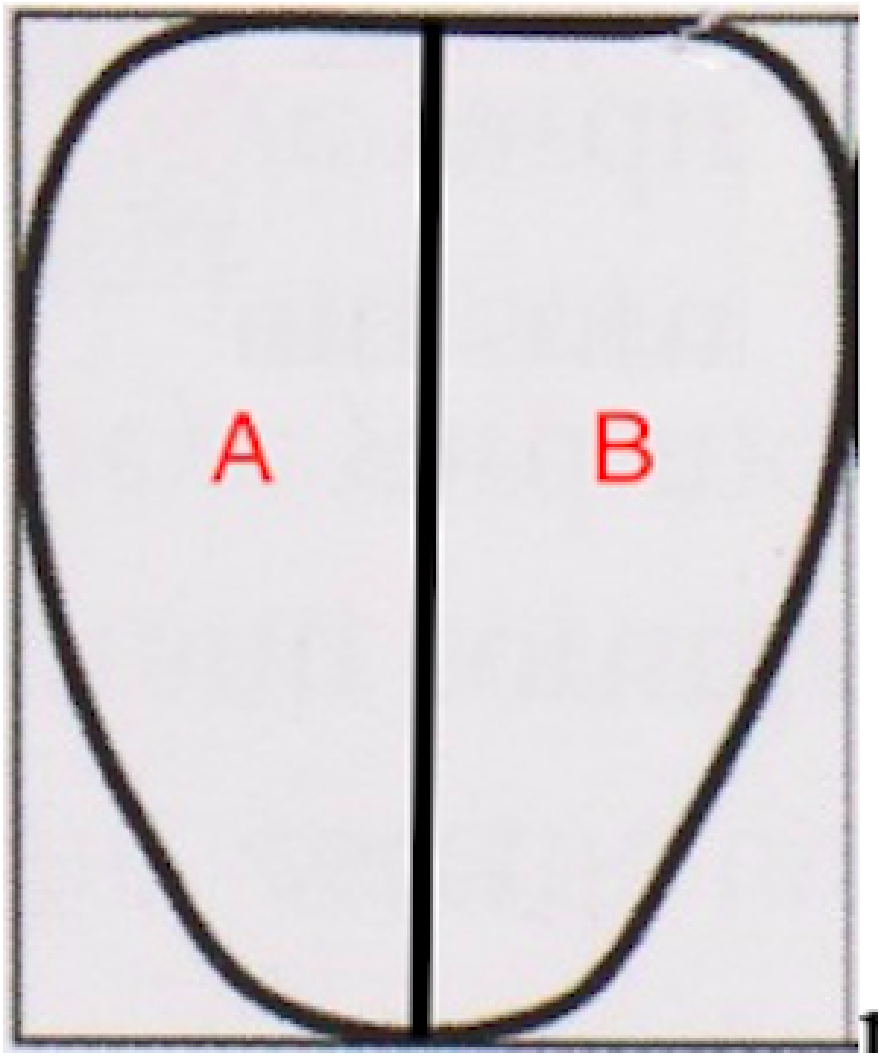

Figure 1: Crown separated into two equal parts from gingival to insizal

2.2 Creation of Initial Enamel Lesions

To create the initial enamel lesion, a demineralization solution was prepared by the Atatürk University Faculty of Pharmacy with a pH of 4.5. The Demineralization Solution was prepared as 0.075 mol l-1 Acetic acid, 0.002 mol 1-1 Ca (from CaCl2), and 0.002 mol 1-1 P (KH2PO4). The left samples were kept in the artificial saliva solution prepared by the Atatürk University Faculty of Pharmacy after demineralization. The prepared samples were held in the demineralization solution for 8 hours per day until 13–20 values were obtained through dignodent measurement and then kept in the dark environment in artificial saliva for the remaining time. The solution was renewed every day. The demineralized teeth were kept in the artificial salivary solution until the experimental materials were applied.

2.3 Application of Remineralization Agents

The teeth' crowns were divided into two, with 80 samples prepared from 40 anterior maxillary teeth (n=80). The samples were randomly distributed among five experimental groups containing the right and left halves of eight teeth in each group (Table 1). The materials and material contents used in our study are presented in Table 2.

Table 1. Practices applied to the A and B halves of teeth according to material groups

Groups	**A – Experimental Groups**	**n**	**B – Control Groups**	**n**
Group 1 :	GC MI Varnish, GC Corp, Tokyo, Japan	8	Demineralization	8
Group 2:	GC Tooth Mousse, GC Corp, Tokyo, Japan	8	Demineralization	8
Group 3:	Remin Pro, Voco, Germany	8	Demineralization	8
Group 4:	Remineralizing gel, R.O.C.S. Trading GmbH, Munich, Germany	8	Demineralization	8
Group 5:	Artificial Saliva (no process)	8	Demineralization	8

Table 2. Materials and material content used in the study.

Materials	Materials Content
MI Varnish	CPP-ACP, 5 % NaF(22 600ppm), ethanol, mint flavor
GC Tooth Mousse	Glycerol, CPP-ACP, D- Sorbitol, CMC-Na, propylene glycol, silicon dioxide, titanium dioxide, Xylitoll , Phosphoric acid, Sodium saccharin, Ethyl p-hydroxybenzoate, Propyl p-hydroxybenzoate, Butyl p-hydroxybenzoate, Flavor, Pure Water
Remin Pro	Hydroxyapatite (HA), NaF (1450 ppm flor), xylitol, ginger extract
ROCS® Medical Mineral Gel	Aqua, Glycerin, Xylitol, Hydroxyethylcellulose, Calcium Glycerophosphate, Polysorbate-20, Aroma, Methylparaben, Magnesium Chloride, Hydroxypropyl Guar
Artificial Saliva	sodium chloride (0.4 g / l), potassium chloride (0.4 g / l), calcium chloride-H2O (0.795 g / l), sodium dihydrogen phosphate-H2O (0.69 g / l), and sodium sulphur-9H2O (0.005 g / l) 1000ml of distilled water

After completed the demineralization process, remineralization materials were applied to the left half of

the teeth (A) according to the manufacturer's recommendations. The right halves of the teeth (B) were subjected to no other processes after demineralization for control purposes and were kept in artificial saliva for the experiment's duration.

2.4 Groups

Group 1) Varnish group with 5% NaF and CPP- ACP (GC MI Varnish, GC): 48 hours before the SEM analysis, the varnish with sodium fluoride and CPP- ACP content was applied once to the surface of the samples. The varnish was left on the teeth for 4 minutes and then removed from the tooth surface with an air-water spray. For the duration of the experiment, the samples were kept in artificial saliva.

Group 2) Paste group with CPP-ACP (GC Tooth Mousse, GC): A paste containing CPP-ACP was applied twice a day (09:00–16:00) with the help of a cotton tip applicator. The paste was then removed from the surface with pressured water after waiting 3 minutes on the left surface. For the duration of the experiment, the samples were kept in artificial saliva.

Group 3) Paste group with NaF, HA, and xylitol (Remin Pro, Voco): Remin Pro was applied to the surface of the samples for 3 minutes, twice a day (09:00–16:00) for 21 days using a cotton tip applicator. The paste was then removed from the surface with pressured water. For the duration of the experiment, the samples were kept in artificial saliva.

Group 4) Gel group with xylitol (R.O.C.S ® Medical Mineral Gel): R.O.C.S® Medical Mineral Gel was applied to the surface of the samples for 3 minutes, twice a day (09:00–16:00) for 21 days using a cotton tip applicator. The gel was then removed from the sample surface after the procedure with pressured water. For the duration of the experiment, the samples were kept in artificial saliva.

Group 5) Control Group (Artificial Saliva): For the experiment's duration, the samples were kept in artificial saliva.

After the material's final applications were completed, the samples were kept in artificial saliva for 24 hours. The teeth then underwent SEM-EDX analyses at the Eastern Anatolia High Technology Application and Research Center (DAYTAM).

2.5 Statistical Analysis

The data were analyzed using the SPSS 20.0 package program. For the EDX assessments, a One-Way ANOVA was performed. In all analyses, a p-value < 0.05 was considered statistically significant.

3 Results

3.1 Results of the EDX Analysis

The EDX analysis results, carried out to evaluate the effect of applied remineralization materials on mineral distributions over the demineralized enamel surface, are presented in Table 3. According to the E.D.X. analysis assessment, the low Ca/P ratio obtained in R.O.C.S. Remineralizing gel (Group 4) material was differed statistically from the other materials (p=0.04) and the control group, including the other teeth halves (p=0.025).

Table 3. Results of the EDX analysis.

Groups	**Experimental Groups Ca/P ratio Mean±SD A**	**Control groups Ca/P ratio Mean±SD B**	**p**
Group 1(GC MI Varnish**)**	1.94±0.670[b]	1.90±0.065	0.919
Group 2 (GC Tooth Mousse**)**	1.88± 0.138[b]	1.87±0.089	0.974

Groups	Experimental Groups Ca/P ratio Mean±SD A	Control groups Ca/P ratio Mean±SD B	p
Group 3 (Remin Pro,)	1.99±0.055[b]	1.78±0.068	0.014*
Group 4 (R.O.C.S®)	1.79±0.035 [a]	1.58±0.239	0.025*
Group 5 (Artificial Saliva)	1.96±0.089 [b]	1.77±0.005	0.022*
P difference	0.04*	0.051	

In the E.D.X. analysis assessments, the Ca/P ratio did not differ statistically significantly compared to GC MI Varnish groups (p=0.919) and G.C. Tooth Mousse groups (p=0.974). In contrast, Group 3 (p=0.014) and Group 4 (p=0.025) differed statistically significantly from the control group. Moreover, a statistically significant difference was noted between the demineralization halves and the healthy enamel halves on which no action was performed in the control group (p=0.022).

3.2 Results of the SEM Analysis

In the SEM analysis evaluations, the different remineralization agents applied to the initial enamel lesions created on the enamel surface were found to develop other morphological appearances on the surface. Figure 2 shows the healthy enamel surface's SEM image, which did not undergo demineralization. It was detected slightly pores between the calcifiedareas on the enamel surface.

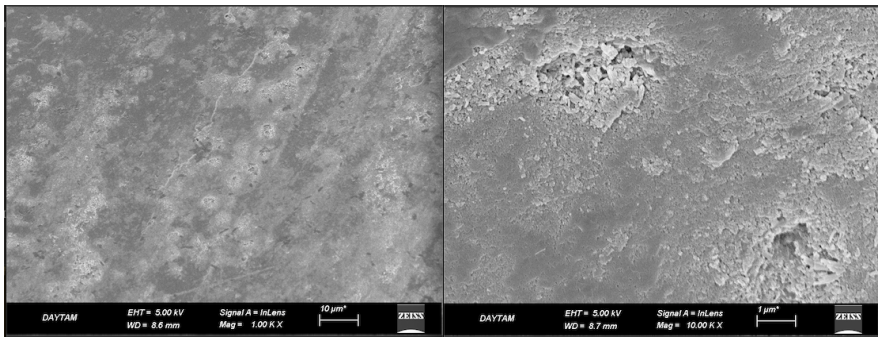

Figure 2 : SEM images of the solid enamel surface of the control group (x100, x1000)

The SEM images of the surface of the demineralized enamel are presented in Figure 3 After demineralization, it was observed that the aprismatic enamel layer on the enamel surface had been removed, and small gaps in the shape of key holes had appeared in the interprismatic regions under x10000 magnification.

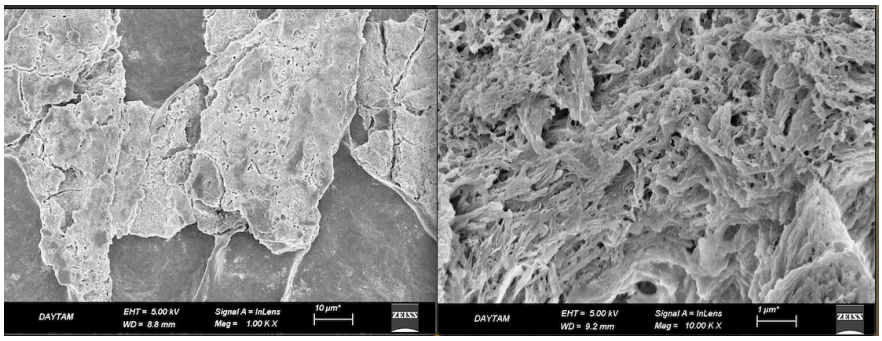

Figure 3: SEM images of demineralized enamel surface (x1000, x10000).

It can be seen that the GC MI Varnish (Group 1) layer applied after demineralization remained on the enamel surface, although small pores can be determined, through which the surface of the enamel can be seen (Figure 4).

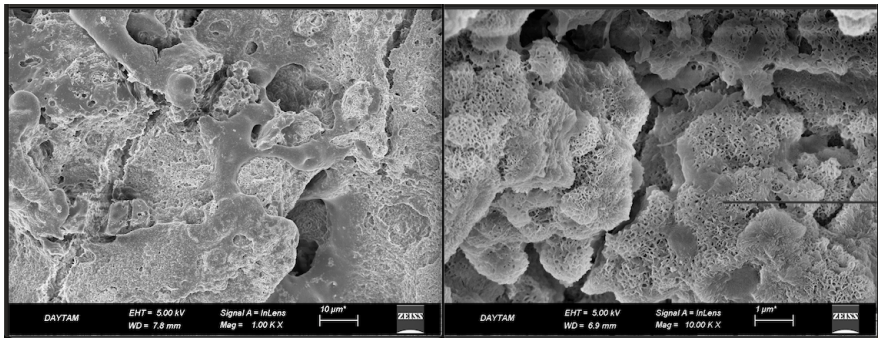

Figure 4: SEM images from Group 1 (x100, x1000), consisting of a GC MI Varnish application to the surface of demineralized enamel.

GC Tooth Mousse (Group 2) was applied after demineralization (Figure 5). The CCP-ACP applied to the porous surface of the demineralized layer has not been wholly lost.

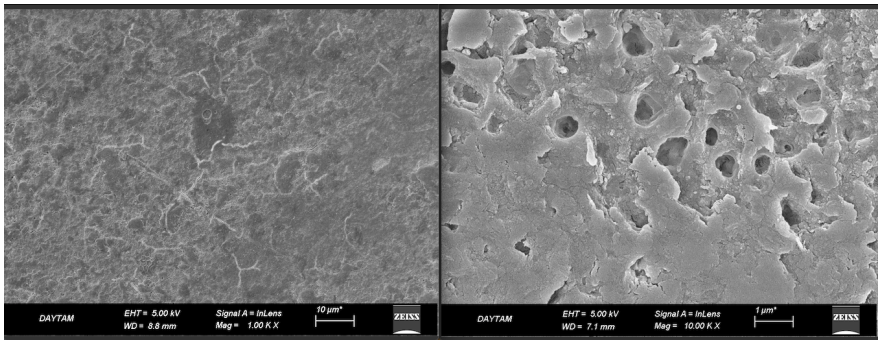

Figure 5.: SEM images of Group 2 formed after the application of GC Tooth Mousse to the demineralized enamel surface (x100, x1000)

SEM images of Group 3, treated with a Remin Pro, in which the remineralization material was applied to the demineralized enamel's surface, are given in Figure 6. It can be observed that the applied material has closed the gaps in the interprismatic area, although the image is not homogeneous.

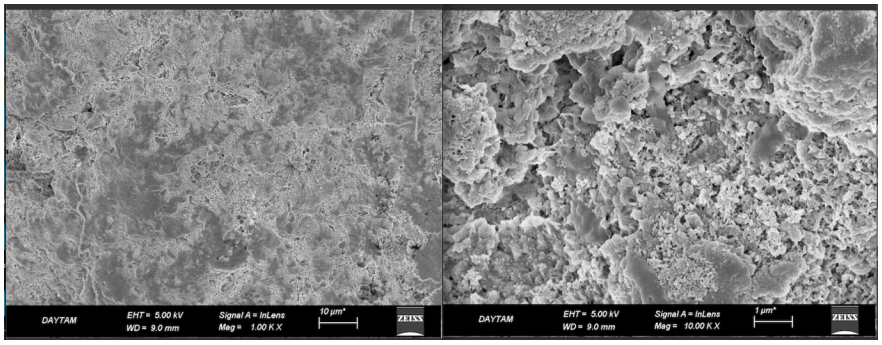

Figure 6. : SEM images of Group 3 (x100, x1000) Remin Pro application to the enamel surface after the demineralization process

R.O.C.S® (Group 4), applied to the surface of the enamel after demineralization (Figure 7). The SEM images show the remains of a pebble-like material on the surface.

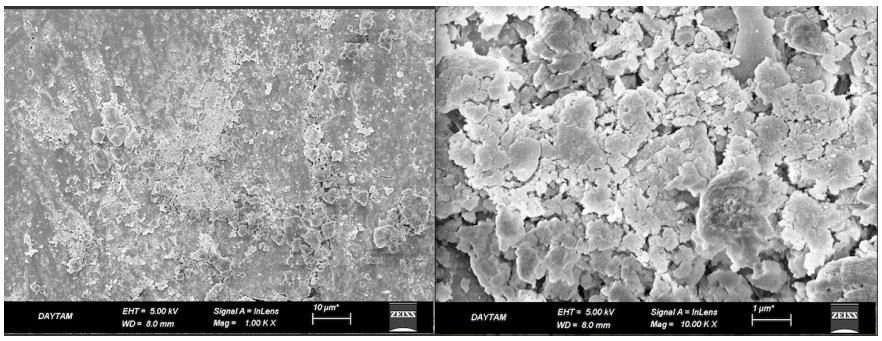

Figure 7.: SEM images of Group 4 in which R.O.C.S®Remineralizing gel was applied to the surface of the demineralized enamel (x100, x1000)

4. Discussion

Initial enamel lesions are bruises caused by mineral loss to the surface, with decay limited only to the enamel tissue, before affecting the deep tissues.[9] Initial enamel lesions have a reversible stage, with a remineralization process.[6]The ideal approach to remineralization is to reconstruct the Ha crystal, as the enamel's inorganic component, [10]and the most commonly used agents for this purpose are fluorides. Clinically applied fluorinated varnish adheres to the enamel's surface and creates a CaF2 layer, creating a physical barrier against acid changes.[1] However, in studies evaluating the effect of fluorides on remineralization, it has been reported that it is impossible to explain the response of the enamel to acid attacks by the presence of F-ions alone. Ca + 2 and PO-4 are needed to create new FAP or FHAP crystals during topical fluoride applications .[12,13] Therefore, no matter how high the concentrations of the topical agents found in the environment, it has been reported that the F ions cannot be adequately utilized if there are insufficient Ca+2 and PO4- when applied to the surface of the enamel. For this reason, an EDX analysis was performed in the present study and evaluated the Ca/P ratio. In the present study, MI varnish (22600 ppm) and Remin Pro (1450 ppm) containing NaF were chosen for different doses, and no difference was identified between them in terms of the Ca/P ratio. While some studies have argued that fluorine is more effective in high doses, other studies have found increased doses to be insignificant, similar to our results.[15] This difference in the findings of studies may be attributed to the fact that from different working designs, and for this reason, alternative flora agents have been developed. It has long been known that dairy products have anti-cariogenic roles, with studies in recent years reporting that this effect is due to the casein phosphopeptide (CPP) found in cheese structures.[16]

In addition to such advantages as not being CPP cytotoxic, being an inorganic ion carrier, being a reliable agent, and supporting the absorption of iron, zinc, selenium Ca^{+2}, particularly from the intestines[17], CPP and ACP also act as a Ca^{+2} - PO^{-4} reservoir by activating the Ca^{+2} and PO^{-4} ions in free form in the plaque fluid as a result of the nanomolecular form[17]. Thanks to , there have been studies indicating that CPP and ACP can prevent demineralization and be involved in remineralization.[18] There was no difference between the Tooth Mouse group and the MI Varnish group containing CPP-ACP in our study, and no difference was found between these groups and the control group, comprising half of the teeth. In a study in which a CPP-ACP agent was applied over different time periods, a higher Ca/P ratio was noted in the long-term groups.[8] In contrast to this study, in the present study's the MI varnish group underwent only one application, 48 hours before measurement, in line with the manufacturer's recommendations. In the Tooth Mouse group, despite the twice a day applications for 21 days, no difference in results could be identified between MI varnish and Tooth mouse. The difference in results may be because, in the other study, the control group enamel samples were not obtained from the same teeth, and the samples used in the control group had different Ca/P ratios. A new and rare remineralization agent, Remin Pro, is a remineralization agent containing Ca, P, HA, xylitol, and F.[10,19] There have been studies indicating that Remin Pro, which contains HA and xylitol, increases enamel microhardness in initial enamel lesions.[20]In the present study, the Remin Pro group was one of the two groups that recorded significant differences from the control group. In remineralization studies in which HA was used alone, it was found to have positive results on the enamel.[21] Also, studies of children who chew Xylitol gum have found it successful in preventing caries.[22] Our results suggest that the co-use of HA, fluoride, and xylitol use may be beneficial in initial enamel lesions, although further studies of this issue are needed. In addition to fluoride, CPP-ACP, HA, and Xylitol applications, R.O.C.S® Medical Mineral Gel, a new mineral gel aimed at increasing the mineral level on the surface of the enamel, has been developed.[9]Another study, which looked at the effect with Lazer, found that it increased the Ca/P ratio for R.O.C.S® Medical Mineral Gel but noted that the difference was not significant.[23] In the present study, the difference in the Ca/P ratio found between the findings associated with R.O.C.S® Medical Mineral Gel and the control group was significant. In contrast, the Ca/P ratio of R.O.C.S® Medical Mineral Gel was the lowest value than the other agents. The conflicting results can be thought to be attributable to the different study designs and sample numbers, and more studies are thus needed to clarify the situation. After half of the teeth were demineralized, the samples were compared with the other halves that had not been subjected to any processes. The latter were found to have higher Ca/P ratios, as would be expected.[24] However, The differences between the agents other than R.O.C.S® Medical Mineral Gel were found to be insignificant. In studies evaluating demineralization and remineralization processes, SEM images provide detailed and sensitive information when used in conjunction with an EDX analysis of the morphological changes that occur on the surface of the enamel.[25] In the present study, SEM images were also taken after the mineral level changes produced by different remineralization agents were evaluated by an EDX analysis. In studies evaluating the activities of florids on the surface of the enamel through SEM, the enamel surface was found to be coated with CaF^2 crystals.[26] In one study, SEM images obtained at different remineralization times were evaluated, and it was seen that the demineralized enamel surface structure transformed from a porous scale-like apatite to a flat apatite.[27]Similar surface properties were observed in the SEM evaluations of CPP-ACP, CPP-ACP + F containing paste, HA, Xylitol, F containing paste and mineral gel, and the control group. It was observed that the surface was covered with a new remineralization layer, which appeared to mask the surface appearance by filling the depressions formed by carving the prism cores. It was further observed that the surface layer that formed consisted of round-shaped globules. Jayarajan et al. evaluated the effectiveness of remineralization on the initial enamel lesions of CPP-ACP with CPP-ACP+F in their work under *in-vitro*conditions.[17] In the CPP-ACP group, the calcification zones of porous structures on the enamel surface were monitored regularly and in detail. The CPP-ACP+F group is similar to the CPP-ACP group, although the calcification was slightly more pronounced. It was found in the study that CPP-ACP and CPP-ACP+F varnish affected the initial enamel lesion, but there was no significant difference between the groups. In these two different SEM studies with varnish with CPP-ACP and CPP-ACP+F, the agents formed a layer of remineralization on the surface of the enamel that increased resistance to demineralization and reported parallel findings to those in the present study.[17,27] There are some limitations to the present

study, which include the lack of a comparison with other remineralization agents, the failure to carry out any other analyses to the SEM/EDX analyses, and the lack of an evaluation of ions other than the Ca/P ratio in the EDX analysis.

The following conclusions were reached in the study:

1. When compared to the demineralized halves of the teeth, CPP-ACP (GC Tooth Mousse) and CPP-ACP+F (MI Varnish) were found to have not affected on the initial enamel lesions.
2. Compared to the teeth' demineralized halves, HA xylitol and F (Remin Pro) were found to have affected on initial enamel lesions.
3. Compared to the teeth' demineralized halves, the mineral gel containing minerals such as Ca and P (R.O.C.S (r) Medical Mineral Gel) may be considered having affected on initial enamel lesions.
4. Further extensive scientific studies are needed to identify the most effective agent for the treatment of initial enamel lesions.

5.References

1. Asokan S, Geethapriya P, and Vijayasankari V. Effect of nonfluoridated remineralizing agents on initial enamel carious lesions: A systematic review. I*ndian Journal of Dental Research* , 2019; 30(2): 282.

2. Talaat DA, Abdelrahman AA, Abdelaziz RH, et al. Effect of Two Remineralizing Agents on Initial Caries-like Lesions in Young Permanent Teeth, An in Vitro Study. *The journal of contemporary dental practice* . 2018; 19(10): 1181-1188.

3. Daas I, Badr S, and Osman E. Comparison between fluoride and nano-hydroxyapatite in remineralizing initial enamel lesion: an in vitro study. *J Contemp Dent Pract.* 2018; 19(3): 306-312.

4. Gonzalez-Cabezas C, and Fernandez C. Recent advances in remineralization therapies for caries lesions. *Advances in Dental Research.* 2018; 29(1): 55-59.

5. Xiang C, Ran J, Yang Q, et al. Effects of enamel matrix derivative on remineralisation of initial enamel carious lesions in vitro.*Archives of Oral Biology.* 2013; 58(4): 362-369.

6. Urquhart O, Tampi MP, Pilcher L, et al. Nonrestorative treatments for caries: systematic review and network meta-analysis. *Journal of dental research.* 2019; 98(1): 14-26.

7. Rallan M, Chaudhary S, Goswami M, et al. Effect of various remineralising agents on human eroded enamel of primary teeth.*European Archives of Paediatric Dentistry* . 2013; 14(5): 313-318.

8. HegdeMN, and Moany A. Remineralization of enamel subsurface lesions with casein phosphopeptide-amorphous calcium phosphate: A quantitative energy dispersive X-ray analysis using scanning electron microscopy: An in vitro study. *Journal of conservative dentistry: JCD.* 2012; 15(1): 61.

9. Wegehaupt FJ, Schleich J,Hamza B, et al. Performance of a newly developed mineral gel system on erosive and erosive/abrasive enamel loss An in vitro study. S*wiss dental journal.* 2018; 127(10): online.

10. Abdelmegid FY, Salama FS, Abouobaid EI, et al. Effect of Remineralizing Agents on Bond Strength of Resin-Composites to Primary Enamel. J*ournal of Clinical Pediatric Dentistry.* 2019; 43(5): 331-336.

11. Llena C, Esteve I, Rodrıguez- Lozano FJ, et al. The application of casein phosphopeptide and amorphous calcium phosphate with fluoride (CPP-ACPF) for restoring mineral loss after dental bleaching with hydrogen or carbamide peroxide: An in vitro study. *Annals of Anatomy-Anatomischer Anzeiger.* 2019; 225: 48-53.

12. Harper DS, Osborn JC, HEfferen JJ, et al. Cariostatic evaluation of cheeses with diverse physical and compositional characteristics.*Caries Research.* 1986; 20(2): 123-130.

13. Zhang Q, Zou J, Yang R, et al. Remineralization effects of casein phosphopeptide-amorphous calcium phosphate crème on artificial early enamel lesions of primary teeth. *International Journal of Paediatric Dentistry.* 2011. 21(5): p. 374-381.

14. Reynolds EC. Calcium phosphate-based remineralization systems: scientific evidence? *Australian dental journal.* 2008; 53(3): 268-273.

15. Zhou C,D Zhang, Y Bai, et al. Casein phosphopeptide–amorphous calcium phosphate remineralization of primary teeth early enamel lesions. *Journal of dentistry.* 2014; 42(1): 21-29.

16. Llena C, Leyda A, and Forner L. CPP-ACP and CPP-ACFP versus fluoride varnish in remineralisation of early caries lesions. A prospective study. *Eur J Paediatr Dent* . 2015; 16(3): 181-6.

17. Jayarajan J, Janardhanam P, and Jayakumar P. Efficacy of CPP-ACP and CPP-ACPF on enamel remineralization-An in vitro study using scanning electron microscope and DIAGNOdent(r). *Indian journal of dental research* . 2011; 22(1): 77.

18. Thanatvarakorn O, Nakashima S, SAdr A et al. Effect of a calcium-phosphate based desensitizer on dentin surface characteristics.*Dental materials journal.* 2013; 32(4): 615-621.

19. Heshmat H, Ganjkar MH, Jaberi S et al. The effect of remin pro and MI paste plus on bleached enamel surface roughness. *Journal of Dentistry (Tehran, Iran).* 2014; 11(2): 131.

20. Salama F, Abdulmegid F, Al-Mutairi F, et al. Effect of Remineralizing Agents on Enamel Surface Roughness of Permanent Teeth: An In Vitro Study. *Journal of Dental and MEdical Sciences.* 2020; 19(4): 47-55

21. Shahmoradi M, Rohanizadeh R, Sonvico F, Ghadiri M, et al. Synthesis of stabilized hydroxyapatite nanosuspensions for enamel caries remineralization. *Australian Dental Journal.* 2018; 63(3): 356-364.

22. Gargouri W, Kammoun R, Elleuche M, Tlili M, et al. Effect of xylitol chewing gum enriched with propolis on dentin remineralization in vitro.*Archives of Oral Biology* , 2020; 112: 104684.

23. Yilmaz N, Baltaci E, Baygin O, Tuzuner T, et al. Effect of the usage of Er, Cr: YSGG laser with and without different remineralization agents on the enamel erosion of primary teeth. *Lasers in Medical Science,* 2020.

24. Ionta FQ, Mendonca FL, de Oliveria GC, Alencar C, et al. In vitro assessment of artificial saliva formulations on initial enamel erosion remineralization. *Journal of dentistry.* 2014. 42(2): 175-179.

25. Simsek H, Coruh M, Cakıcı F, et al. Investigation of mineral content of root canal dentin after the application of various antibiotic paste using energy-dispersive X-ray detector. Microscopy research and technique. 2019; 82(2): 144-148.

26. Zhou J, Watanabe S, Watanabe K, Wen LY, et al. In vitro study of the effects of fluoride-releasing dental materials on remineralization in an enamel erosion model. *Journal of dentistry.* 2012; 40(3):255-263.

27. Chen CL, Parolia A, Pau A, et al. Comparative evaluation of the effectiveness of desensitizing agents in dentine tubule occlusion using scanning electron microscopy. *Australian dental journal.* 2015; 60(1):65-72.

Hosted file

`Table -1.pdf` available at https://authorea.com/users/393004/articles/506743-evaluation-of-the-effects-of-different-remineralization-agents-on-initial-enamel-lesions-by-scanning-electron-microscope-and-energy-distributed-x-ray-analysis

Hosted file

`Table-2.pdf` available at https://authorea.com/users/393004/articles/506743-evaluation-of-the-effects-of-different-remineralization-agents-on-initial-enamel-lesions-by-scanning-electron-microscope-and-energy-distributed-x-ray-analysis

Hosted file

Table-3.pdf available at https://authorea.com/users/393004/articles/506743-evaluation-of-the-effects-of-different-remineralization-agents-on-initial-enamel-lesions-by-scanning-electron-microscope-and-energy-distributed-x-ray-analysis