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Effect of Remineralizing Agents on Enamel Surface Roughness of Permanent Teeth: An In Vitro Study

Fouad Salama[1], Faika Abdelmegid[2], Faisal Al-Mutairi[3], Abdulrahman Al-Nasrallah[3], Mohammed Al-Sharhan[3], Nassr AlMaflehi[4], Ihab Moussa[5]

[1]Professor Pediatric Dentistry, University of Detroit Mercy, School of Dentistry, USA; Former Professor, Department of Pediatric Dentistry and Orthodontics, College of Dentistry, King Saud University, Saudi Arabia
[2]Former Professor, Department of Oral Medicine and Diagnostic Sciences, College of Dentistry, King Saud University, Riyadh, Kingdom of Saudi Arabia
[3]Former Dental Interns, College of Dentistry, King Saud University, Riyadh, Kingdom of Saudi Arabia
[4]Department of Periodontics and Community Dentistry, College of Dentistry, King Saud University, Riyadh, Kingdom of Saudi Arabia
[5]Department of Restorative Dental Sciences, College of Dentistry, King Saud University, Riyadh, Kingdom of Saudi Arabia
Corresponding Author: Fouad Salama

Abstract

Purpose:*The objective of this study was to compare the effect of three remineralizing agents [casein phosphopeptide-amorphous calcium phosphate (CPP-ACP - MI Paste), casein phosphopeptide-amorphous calcium phosphate with fluoride (CPP-ACPF - MI Paste Plus), and hydroxyapatite (Remin Pro) on surface roughness of sound and demineralized dental enamel of permanent teeth in vitro.*

Methods: *Sound and demineralized enamel were divided into 8 groups according to the remineralizing agents and positive and negative controls (artificial saliva).The initial readings of surface roughness at baseline (T1) and after application of the three remineralizing agents for 30 minutes and seven and half hours (T2 and T3) were recorded.*

Results: *Demineralized enamel at T2, showed significant difference between the control and MI Paste (P=0.019) as well as MI Paste and MI Paste Plus (P=0.004). Demineralized enamel at T3, showed significantdifference between the control and Remin Pro (P=0.035) as well as MI Paste (P=0.0001). In addition, there was significant difference between the Remin Pro and MI Pasteand MI Paste Plus (P=0.0001) as well asMI Paste and MI Paste Plus (P=0.0001).*

Conclusion: *There was significant difference between surface roughness (Sa) of the sound and demineralized enamel of the control and the three tested remineralizing agents after 8 hours exposure to the reminralizing agents and artificial saliva.MI Paste did show significant effect on sound enamel of permanent teeth after the second application of the reminralizing agents while MI Paste Plus with fluoride, Remin Pro and artificial saliva did not.*

Keywords*: Surface Roughness, Remineralizing Agents, Enamel, Permanent Teeth*

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I. Introduction

The new tooth remineralization technologies include compounds with or without the additional or synergistic effects of fluoride to enhance the remineralization process and improve the mechanical properties of the demineralized substrate [1,2]. Commonly used remineralization materials are unstabilized amorphous calcium phosphate (ACP), casein phosphopeptide stabilized amorphous calcium phosphate (CPP-ACP), casein phosphopeptide-amorphous calcium phosphate fluoride (CPP-ACPF), a bioactive glass containing calcium sodium phosphosilicate, hydroxyapatite that contains calcium, phosphate, and tricalcium phosphate fluoride (TCP-F) [3]. As remineralization materials depend on phosphate and calcium compounds, their influence is mostly relying on the augmentation of the ability of saliva to remineralize mineral loss.[3] CPP-ACP complexes have been shown to inhibit demineralization of enamel and stimulate remineralization of early subsurface enamel lesions [4]. CPP-ACP was commercialized as Recaldent™ and added to sugar-free chewing gums in some countries such as America and Australia as well as used as remineralizing pastes [4]. The CPP-ACP complex is commercially available in mousse or paste form such as Tooth Mousse/MI Paste and Tooth Mousse Plus/MI Paste Plus (GC America Inc.). MI Paste is without fluoride while MI Paste Plus contains CPP-ACP and fluoride (900 ppm). Several studies have reported the effectiveness of the CPP-ACP technology in inhibiting demineralization and stimulating remineralization of enamel and dentin [1, 2]. It has been suggested that remineralizing agents have anti-erosive and anticariogenic properties [5]. When placed on the human enamel surface it can interact with hydrogen ions, form calcium hydrogen phosphate, which releases calcium and phosphate ions, which prevents the acid dissolution and protect the enamel [5]. Remin Pro is another type of remineralizing agent, which in contrast to CPP-ACP products contains calcium, phosphate in the hydroxyapatite form [6]. In addition, fluoride and Xylitol have also been included in this product [6]. Remin Pro contains hydroxylapatite particles much similar to phosphate and calcium ions in CPPACPF that are deposited on the bleached enamel surface and increase the microhardness of teeth [7]. Overall, the remineralizing agents such as Remin Pro and MI Paste Plus decrease the surface roughness after bleaching [7].

The significance of the surface of enamel in caries progression and remineralization is critical. Moreover, roughness is essential property of teeth, which influence the attachment of foreign materials to their surfaces. Surface roughness is one of the commonly used tests to assess the effect of different materials on dental hard tissues[8] and is well recognized for quantifying surface texture [9]. To our knowledge, limited studies have compared the influence of remineralizing agents on roughness of sound and demineralized enamel of permenant teeth and no prior investigation used a 3D optical noncontact surface profiler to measure their roughness. Thus, the objective of this investigation was to compare the influence of three remineralizing agents CPP-ACP (MI Paste), CPP-ACPF (MI Paste Plus), and hydroxyapatite (Remin Pro) on surface roughness of sound and demineralized enamel of permenant teeth *in vitro*. The tested null hypothesis was that there is no differences between surface roughness of sound and demineralized enamel of permenant teeth after application of different remineralizing agents.

II. Materials And Methods

Forty-eight permanent freshly extracted molar teeth with intact and sound buccal enamel surfaces and on visual inspection devoid of any restorations, enamel cracks, caries, erosion, white spot lesion, or hypoplastic were collected and used in this investigation. The teeth had been thoroughly cleaned of debris and soft tissues attached to the surfaces and then stored in 0.1% thymol solution for 48 hours before use. The roots were separated from their crowns at the cemento-enamel junction using a low speed water-cooled diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA). Then, the crowns had embedded horizontally in the self-cure acrylic resin blocks (Duralay; Reliance Dental C., Worth, IL, USA) in a way that the buccal surface is flat. The exposed enamel windows were cleaned and polished with a low-speed rubber cup and slurry of non-flouridated pumice, washed for 30 seconds and dried for 10 seconds with oil-free air spray. Each buccal surface was divided into two sections using a low speed water-cooled diamond saw. The specimens prepared were randomly assigned using simple randomized sampling into eight groups of 12 samples each. The power sample was calculated at level of significance 0.05 and estimated standard deviation = 1 with maximum difference 3 and the sample size from each group was determined to be at least 6. One group acted as the positive control group (Sound enamel/no demineralization) and a negative control group (Demineralized enamel) [10]. The other experimental sound and demineralized enamel groups were assigned to be treated by the three remineralizing agents. Different groups that were used in this investigation are presented in Table 1. Where applicable, the demineralization of enamel was completed by the application of the demineralizing solution, which was prepared similar to a modification of the methods described by Patil and coworkers[3] as follows: 2.2mM calcium chloride (CaCl.2HO) 2.2mM monosodium phosphate (NaHPO.7HO) 0.05M lactic acid. The final pH was adjusted to 4.5 with 50% sodium hydroxide (NaOH). All the samples were immersed into a glass container containing 50ml of demineralizing solution for a period of 72 hours at 37°C. After 72 hours of incubation in the demineralizing solution, the teeth were washed with distilled water. Each sound and demineralized enamel group was placed in glass containers containing 50 ml artificial saliva (Pickering Laboratories, Inc., Mountain View, California, USA) withpH of 6.8 until used.

The baseline/initial readings of surface roughness in microns (μm) at time 1 (T1) were recorded for each specimen. Application of the assigned remineralizing agent: MI Paste, MI Paste Plus and Remin Pro to the experimental groups was completed. Each specimen was dried with a cotton roll, and a thin layer of the assigned remineralizing agent was applied to the enamel surfaces using a small brush and surfaces were kept wet with re-application every 15 minutes with total time of 30 minutes (equal to 3 minutes application for 10 days). After the application of the remineralizing agents, all specimens were placed in the artificial saliva for 24 hoursbefore the readings of surface roughness at time 2 (T2) were recorded similar to initial readings. Then, specimens were stored in artificial saliva for 24 hours. Each specimen was then dried with a cotton roll and a thin layer of the assigned remineralizing agent was applied similar to previous application using a small brush for seven and half hours (equal to 3 minutes application for 150 days). After the application of the remineralizing agents, all specimens were placed in the artificial saliva for 24 hoursbefore the reading of surface roughness (T3) were recorded similar to initial readings. The specimens in the positive and negative control groups were kept in the artificial saliva throughout the experiment.

The reading of surface roughness was recorded for all the specimens after air-drying with gentle jets of oil-free compressed air using a 3D optical noncontact surface profiler. The surface roughness {Sa = Arithmetic mean height}, {Sp = Maximum peak height}, and {Sv = Maximum valley depth}of the enamel specimens were analyzed with the 3D optical noncontact surface profiler (Contour Gt-K1 optical profiler, Bruker Nano, Inc., Tucson, AZ, USA) based on noncontact scanning interferometry to evaluate roughness of each surface. The objective standard camera 1.0X has a magnification 5X. For each enamel section, the profile meter scanned area (3 measurements in different directions) was approximately 1.3 x 1.0 mm^2 and had situated at the center of each surface. Multi-Core Processor with Vision64™ software for accelerated 3D surface measurement and analyses were used for image transfer (Bruker Nano Surface Division, Inc., Tucson, AZ, USA). Three-way ANOVA and apost hoc multiple comparison analysis as well as a paired t-test were used to compare and evaluate interactions between the remineralizing agents and different groups and time. All statistical analyses were set with a significance level of $P<0.05$. The statistical analysis was carried out with SPSS V21.0 (SPSS Inc., Chicago, Ill, USA).

III. Results

The mean and standard deviation of the surface roughness {Sa=Arithmetic mean height} of enamel of prermanent teeth following application of different remineralizing agents andthe positive and negative control groupsat the three tested times is presented in Figure 1. The highest surface roughness was recorded at T2 for sound enamel treated with MI Paste Plus (0.410±0.322). While the lowest surface roughness was recorded at T3 for sound enamel treated with MI Paste (0.243±0.062). Figures 2 and 3 showing the Sp (Maximum peak height) and Sv (Maximum valley depth) respectively.

ANOVA showed significant differences (P=0.000001) of surface roughness (Sa) at each tested time (T1, T2, and T3) for the sound and demineralized enamel and application of different remineralizing agents and control (Table 2). For sound enamel at T1, apost hoc multiple comparison analysis showed significant difference between the control and Remin Pro as well as MI Paste (P=0.0001). In addition, there was significant difference between the Remin Pro and MI Paste (P=0.002) and MI Paste Plus (P=0.003) as well asMI Paste and MI Paste Plus (P=0.0001) (Table 2). For demineralized enamel at T1, apost hoc multiple comparison analysis showed significant difference between the control and Remin Pro (P=0.001). In addition, there was significant difference between the Remin Pro and MI Paste and MI Paste Plus (P=0.0001) (Table 2). For sound enamel at T2, apost hoc multiple comparison analysis showed significant difference between the control and Remin Pro (P=0.001). In addition, there was significant difference between the Remin Proand MI Paste Plus (P=0.021) (Table 2). For demineralized enamel at T2, apost hoc multiple comparison analysis showed significant difference between the control and MI Paste (P=0.019) as well as MI Paste and MI Paste Plus (P=0.004) (Table 2). For sound enamel at T3, apost hoc multiple comparison analysis showed only significant difference between Remin Pro and MI Paste (P=0.002) (Table 2). While, for demineralized enamel at T3 showed significant difference between the control and Remin Pro (P=0.035) as well as MI Paste (P=0.0001). In addition, there was significant difference between the Remin Pro and MI Pasteand MI Paste Plus (P=0.0001) as well asMI Paste and MI Paste Plus (P=0.0001) (Table 2).

Comparison between sound and demineralized enamel after application of the remineralizing agents and control at T1 and T3 is presented in Table 3. There was significant difference between Sa of the sound enamel of MI at T1 and T3 as well as significant difference between Sa of the demineralized enamel of the control, Remin Pro, MI Paste, and Paste Plus at T1 and T3.

IV. Discussion

The null hypothesis of this *in vitro* study was rejected because there were differences between surface roughness of sound and demineralized enamel of permanent teeth after application of different remineralizing agents. In the present study, we used demineralizing solution, which was prepared similar to a modification of the methods described by Patil and coworkers [3], and enamel samples were immersed into a glass container containing 50ml of demineralizing solution for a period of 72 hours at 37°C. Other studies used three hours as the period for demineralization in the pH cycling phase [10, 11] to simulate the duration of demineralization that can occur in the oral cavity [12]. However, it is relevant to emphasize that there are numerous dissimilarities between cycling models and *in vivo* conditions. The pH-cycling model do not entirely simulate the oral conditions where the pH fluctuates frequently, and the levels attained depend upon the individual’s eating habits, oral hygiene practices, fluoride usage, and the composition and quality of saliva and biofilm [11]. Thus, it would be beneficial if the remineralizing agents tested in the present study also will be evaluated *in vivo*. In the present study, the total time of application of remineralizing agent was 8 hours. A study evaluated remineralization efficacy of stannous fluoride (SnF_2), CPP-ACPF and calcium sucrose phosphate

(CaSP) concluded that all remineralizing agents showed improved surface remineralization, however, complete remineralization did not occur within 7 days [13]. Therefore, the application of remineralizing agents for 8 hours in the present study may not test long-term effect of remineralizing agent on surface roughness. As far as the authors are aware, little information is known regarding the surface roughness of enamel of permanent teeth after application of different remineralizing agents. In addition, little is known about their influence on the long-term, which was attempted to test in this study with total time of 8 hours application.

Remineralization concept is based on compensation of lost minerals from enamel tooth structure by improving the natural ability of saliva to remineralize enamel surfaces [3]. A study showed remineralization when artificial saliva was used but it was least in comparison to other groups [15]. In this study, we used artificial saliva. Previous studies have shown that artificial saliva has no effect on the microhardness and surface roughness of enamel [7, 14]. The present study showed that there was significant difference between Sa of the sound enamel of MI and demineralized enamel of the control, Remin Pro, MI Paste, and MI Paste Plus after 8 hours exposure to the reminralizing agents and artificial saliva. Additionally, in the present study, the highest surface roughness was recorded at T2 for sound enamel treated with MI Paste Plus, which may indicate increase roughness of the surface after 30 minutes of application compare to control. In addition, the lowest surface roughness was recorded at T3 for sound enamel treated with MI, which may indicate decrease roughness of the surface after 8 hours of application compare to control.

Surface roughness governs the quality, color, buildup of plaque and performance of different surfaces and structures in the oral cavity [16]. In the present study, the permanent teeth showed significant differences of surface roughness (Sa) at (T1) for the sound and demineralized enamel and application of different remineralizing agents and control. This difference could be related to the difference in enamel structure. The enamel surface presents a natural roughness due to the presence of Retzius grooves, pits and small defects, as well as mineral deposits occur in the oral environment [17]. The enamel surfaces used in this study were unground and only the middle third of buccal surface were used to have comparable zone from different teeth with possible similar physical and chemical characteristics. Moreover, the surface of enamel was polished with pumice, which may slightly increase the roughness as reported by another study [17]. Furthermore, there are some influence of enamel structural on the properties of the surface (roughness and hardness) such as dissimilarities in the alignment of enamel prisms and sheath [18].Additionally, the differences in the surface roughness of enamel may be due to the anisotropic structure of enamel and the chemistry of the surface which influence the properties such as more mineralized surface (~9%) than inner enamel after eruption [19].A study evaluated surface roughness of freshly bleached enamel surface exposed to various surface treatments such as fluoride and other remineralizing agents (MI Paste Plus and Remin Pro) found that there was no difference between surface roughness of MI Paste Plus and Remin Pro groups and the surface roughness was decreased compared to the initial enamel surface roughness [7]. In the present study, there was significant difference between Sa of the sound enamel of MI Paste as well as between the demineralized enamel of the control, Remin Pro, MI Paste, and MI Paste Plus at T1 and T3. A study evaluated topical application of CPP-ACP to bleached enamel demonstrated an improvement in surface hardness and decrease in enamel surface roughness [20]. A reduction of surface roughness can be accomplished with remineralization materials such as (CPP-ACP) which will result in a smoother surface resulting in reduced bacterial adhesion, colonization and demineralization [21]. A study of surface roughness of sound enamel surfaces were measured before and after bleaching and application of MI Paste Plus, Remin Pro, and natural saliva showed significant reduction of surface roughness and there was no difference between MI Paste Plus and Remin Pro [22]. In contrast, a study in which bleached enamel surfaces were treated with MI Paste showed that surface roughness neither increase nor decrease [7]. This may be due to different measurement methods used to measure surface roughness of enamel surface. In this study, non-contact optical profiler analysis was used to analyze the surface roughness (Sa, Sv, and Sp) in micrometer. Measurements were obtained from three different directions in the vertical line along the buccal surface of all enamel specimens. There is no agreement about reference data on the limit roughness below which the bacteria would not adhere [23]. The most commonly mentioned limit of surface roughness (Ra) is below 0.2 μm for adherence of dental biofilm [24] and increase of roughness above this value lead to accumulation of bacteria [25]. However, the aforementioned investigations were not performed on enamel surface, but on artificial materials such as cellulose acetate. Maybe it is more accurate to say, that the number depends on the species of bacteria. The surface of enamel is extremely complex with different irregularities, which permits bacterial colonization [24]. The greater the level of magnification during measurement of roughness, the lower Ra or Sa values measured for the same surface. Thus, comparisons between surface roughness data of different studies have to be taken with thoughtfulness due to differences in methods and settings of surface analysis as well as tested surfaces. No study reported human enamel three-dimensional roughness measured at a similar magnification has been published for comparisons. Furthermore, it is not possible to compare roughness values obtained with contact profilometer along one line of the specimen with those values obtained with the non-contact optical interferometers as surface area. As measurement of surface roughness determined by measurement method, the research protocol for roughness is vital [26].A contact profilometer with a stylus that moves in line is used for the quantitative investigation of roughness, may induce misconception due to holes on the surface, and may injury enamel because of its contact with the surface [17]. Other instruments are available to measure roughness at a much higher resolution and over a larger area such as non-contact optical interferometers and atomic force microscopes (AFM). In this study, the optical interferometry noncontact profilometer was used to measure surface roughness. Compared with a stylus profilometer, the optical interferometry noncontact profilometer is faster, nondestructive, and allow repeatability. In addition, it provides a larger field and does not need sample preparation in comparison with AFM [27]. Optical profilers measure roughness (Sa) of a selected micro area at a high spatial resolution with no contact with the specimen. Sa is a surface roughness and for technical surfaces, the relationship between Ra and Sa is 1.25; however, this rule does not have to apply to biological specimen [27].

The adhesive CPP part of the CPP-ACP/ACPF complex binds readily to the enamel and biofilm, providing calcium and phosphate ions exactly where they are needed [4, 7]. The calcium and phosphate ions leave the CPP complex, enter into enamel rods, and increase hydroxyapatite crystals density [4, 28]. It has been reported that addition of fluoride to CPPACP could give a synergistic effect on enamel remineralization [29]. It has been proposed that the remineralization mechanism of CPP-ACP involves localization of ACP at the tooth surface, which buffers free calcium and phosphate ions. By maintaining a state of supersaturation with respect to the hydroxyapatite, these ions depress demineralization and promote remineralization [30].However, the remineralization effect of CPP-ACPF with fluoride was found to be superior to that of CPP-ACP alone. The use of the ACP stabilized CPP system (CPP-ACP) in comparison with ACP used alone demonstrated that CPP-ACP was more effective because it provides a higher amount of available calcium and phosphate ions reservoir which makes it more effective in remineralization, and multiple scientific researches provide that it can even remineralize enamel subsurface and early caries lesions [31].

In general, the solubility of enamel to acid solutions is a function of the chemical content and degree of porosity in the tissue [32-34]. The degree of porosity in enamel explains the differences in demineralization and the tendency to dissolution of enamel [35, 36]. The higher degree of porosity leads to an increase in permeability in the enamel and is caused by a higher interprismatic fraction (interprismatic area related to intraprismatic area) [35]. How large impact the differences of the degree of porosity in the enamel have in demineralization *in vivo* is not yet known [34]. In addition, a study reported that the enamel thickness as numerical density of enamel rods is important factor in demineralization [37]. The chemical content and mineralization of enamel are known to vary between different teeth [36]. The degree of mineralization and chemical content of enamel seem to be of importance for the diffusion rate [35]. In the present study, demineralization was standardized for 72 hours. But it may not produce the same demineralization as previous study reported differences in mechanical properties of enamel, changes related to tooth age, drug effects, absorbed fluoride content, orientation and density of hydroxylapatite crystals, moisture of specimens, and methodology of studies could affect enamel demineralization [39].

The results of this investigation should consider the limitations of the study, including its *in vitro* setting and application of the tested remineralizing agents for only 8 hours, which may not be enough to simulate the cumulative long-term effect *in vivo*. The results may be different if we immersed the tested materials in the remineralizing agents for more time. In addition, the clinical condition in the mouth is not easy to mimic in the laboratory [40].Surface roughness *in vitro* may be different when compared to the dynamic system in the oral cavity *in vivo* and therefore, direct extrapolations to clinical conditions must be exercised with caution [41]. However, in this *in vitro* study, standardization of experimental conditions was advantage and the results demonstrated a clear correlation between surface roughness of enamel of permanent teeth and application of the remineralizing agents and artificial saliva. Moreover, it is clear that change in tooth surfaces is a complex process that can be assessed in many ways and no technique allows for the comprehensive evaluation of a tooth surface, and each technique has its own limitations [42].Moreover, the enamel specimens in our study might not have the same quality despite the fact that the same areas of enamel were used to have comparable zone from different teeth with possible similar physical and chemical characteristics. Furthermore, the present study was performed in the absence of an oral microbial environment or plaque accumulation on the tooth surfaces, which may have affected the results [43].

V. Conclusions

Under the experimental conditions and within the limitations of this *in vitro* study, the following conclusions can be made:The reminralizing agents tested in this study showed significant differences on the surface roughness (Sa) of the sound and demineralized enamel at each tested time (T1, T2, and T3).The lowest surface roughness/smoothest (Sa) was recorded at T3 for sound enamel treated with MI Paste.There was significant difference between Sa of the sound enamel with MI Paste and demineralized enamel of the control, Remin Pro, MI Paste, and MI Paste Plus after 8 hours exposure to the reminralizing agents and artificial saliva.

MI Paste did show significant effect on sound enamel after the second application while MI Paste Plus with fluoride and Remin Pro did not.

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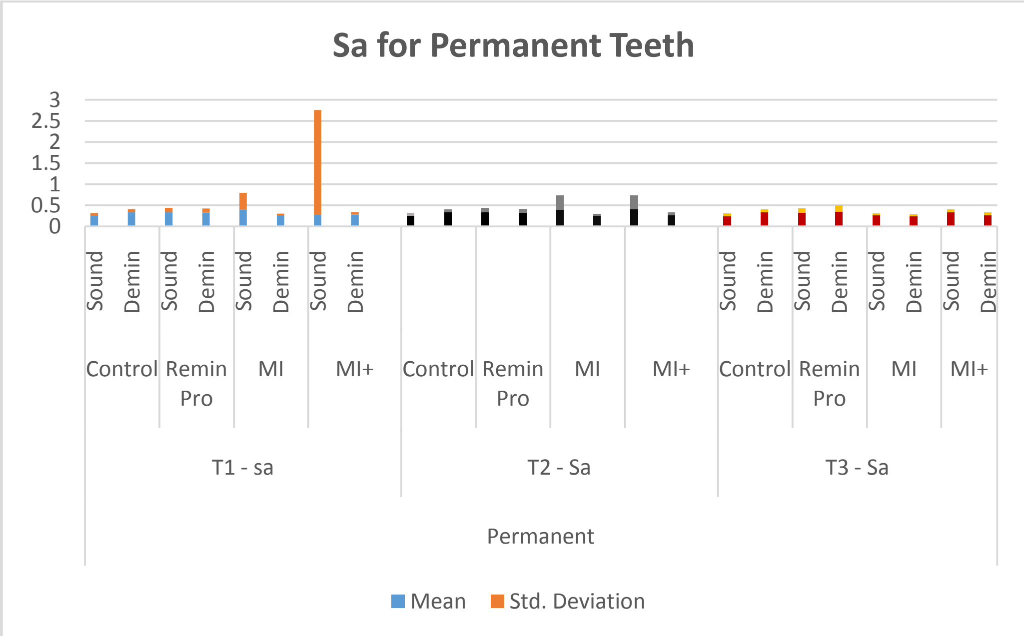

Figure 1. Mean and standard deviations of Sa of permanent teeth following application of different remineralizing agents and control at the three tested times.

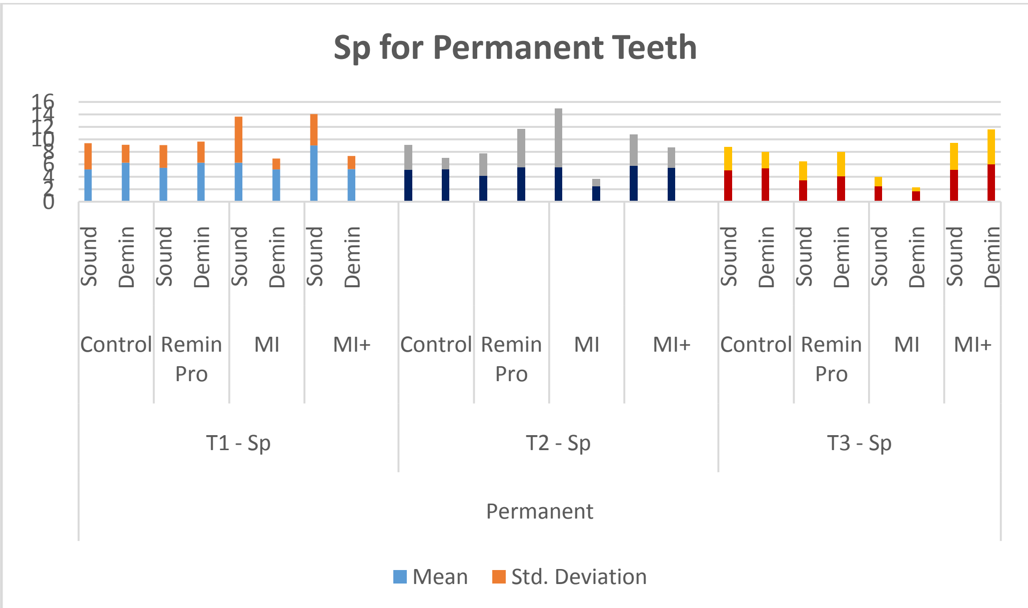

Figure 2. Mean and standard deviations of Sp of permanent teeth following application of different remineralizing agents and control at the three tested times.

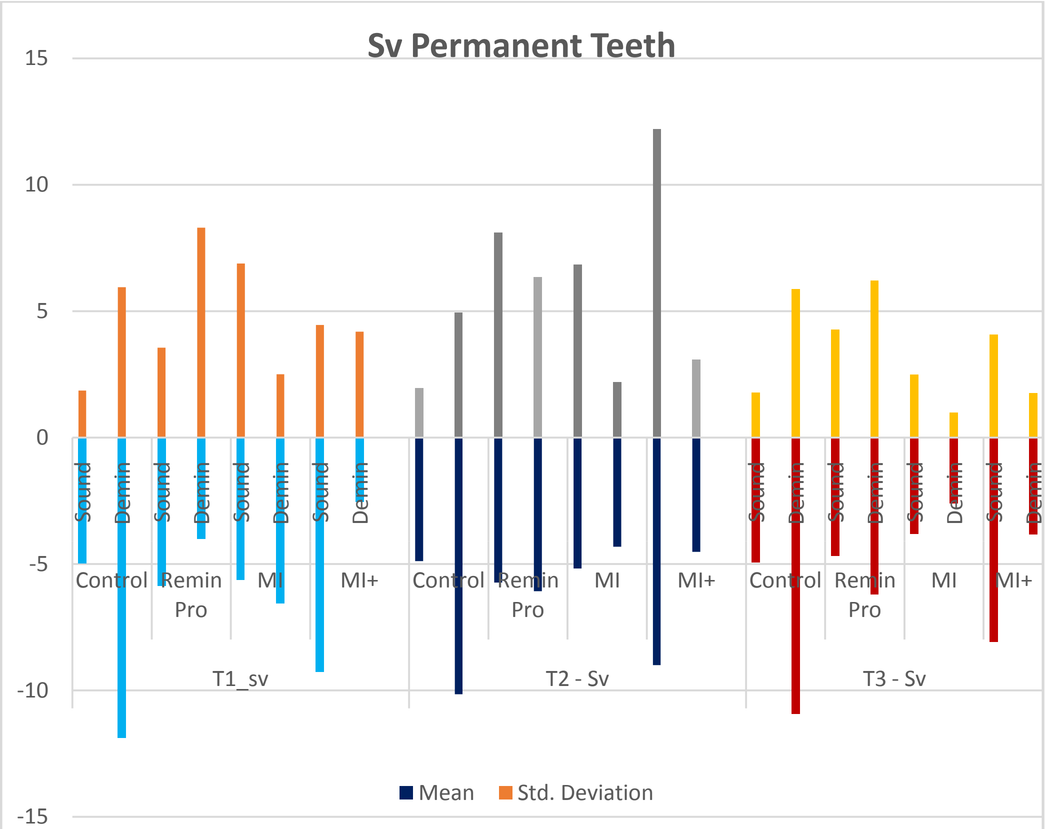

Figure 3. Mean and standard deviations of Sv of permanent teeth following application of different remineralizing agents and control at the three tested times.

Table 1. Distribution of different groups according to surface treatment of permanent teeth enamel and remineralizing agents.

Group Number	Status of Enamel	Remineralizing Agent
1	Sound/No Demineralization - Positive control	None
2	Demineralized - Negative control	None
3	Sound	MI Paste
4	Sound	MI Paste Plus
5	Sound	Remin Pro
6	Demineralized	MI Paste
7	Demineralized	MI Paste Plus
8	Demineralized	Remin Pro

Table 2. Comparison and significance of the arithmetic mean height (Sa) of sound and demineralized enamel following application of remineralizing agents and control at the three tested time periods

Time	Enamel	Remineralizing Agent	ANOVA (P value)	Multiple Comparison Test			
				Control	Remin Pro	MI Paste	MI Paste Plus
T1	Sound	Control	0.000001*	1	0.0001*	0.0001*	0.338
		Remin Pro			1	0.002*	0.003*
		MI Paste				1	0.0001*
		MI Paste Plus					1
	Demin	Control	0.000001*	1	0.001*	0.980	1.000
		Remin Pro			1	0.0001*	0.0001*
		MI Paste				1	0.998
		MI Paste Plus					1
T2	Sound	Control	0.000001*	1	0.001*	0.428	0.124
		Remin Pro			1	0.163	0.021*

		MI Paste				1	1.000
		MI Paste Plus					1
	Demin	Control	0.000001*	1	0.227	0.019*	0.745
		Remin Pro			1	0.202	0.288
		MI Paste				1	0.004*
		MI Paste Plus					1
T3	Sound	Control	0.000001*	1	0.822	0.084	0.989
		Remin Pro			1	0.002*	0.697
		MI Paste				1	0.805
		MI Paste Plus					1
	Demin	Control	0.000001*	1	0.035*	0.0001*	0.985
		Remin Pro			1	0.0001*	0.0001*
		MI Paste				1	0.0001*
		MI Paste Plus					1

* Significant

Table 3. Comparison between sound and demineralized enamel after application of the remineralizing agents and control at T1 and T3.

Enamel	Remineralizing Agent	T1	T3	P-Value
Sound	Control	0.248	-0.807	0.331
	Remin Pro	5.128	-2.214	0.221
	MI Paste	-4.932	3.255	0.0001*
	MI Paste Plus	1.186	0.565	0.763
Demin	Control	2.620	-6.781	0.003*
	Remin Pro	-8.151	0.272	0.0001*
	MI Paste	1.314	5.571	0.0001*
	MI Paste Plus	1.980	-5.239	0.0001*

* Significant

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