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Research Article

Effect of Remineralizing Agents on Enamel Surface Roughness of Primary Teeth: An *In-Vitro* Study

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Abstract

Statement of Problem: Different new tooth remineralization agent for caries control to enhance the remineralization process and improve the mechanical properties of the demineralized substrate. Studying the influence of remineralizing agents on roughness of sound and demineralized enamel of primary teeth of critical importance in clinical dentistry.

Purpose: The objective of this *in vitro* 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 enamel of primary teeth *in vitro*.

Materials and Methods: Sound and demineralized enamel were divided into 8/groups according to the remineralizing agents and control. The initial readings of surface roughness at baseline (T1) and after application of the three remineralizing agents for 30 minutes and seven and a half hours (T2 and T3) were recorded. The surface roughness {Sa = Arithmetic mean height}, {Sp = Maximum peak height}, and {Sv = Maximum valley depth} of the enamel specimens were analyzed.

Results: Sa for sound enamel after 8 hours application to Remin Pro was statistically significantly different from control, MI Paste, and MI Paste Plus. While for demineralized enamel after application of MI Paste was statistically significantly different from control while MI Paste Plus was statistically significantly different from Remin Pro and MI Paste. The lowest Sa at T3 was recorded for sound enamel after application of MI Paste.

Conclusion: There was significant difference of Sa for the sound and demineralized enamel of primary teeth at each tested time (T1, T2, and T3) after application of the remineralizing agents and artificial saliva.

Keywords: *Surface Roughness; Remineralizing Agents; Enamel; Primary Teeth*

Introduction

Demineralization of enamel, appearing as white spot lesions and results in the dissolution of apatite crystals and the net loss of calcium, phosphate, and other ions from the tooth [1]. The objective of contemporary dentistry is to manage non-cavitated caries lesions non-invasively through remineralization in an attempt to prevent disease progression [1]. The significance of the surface of enamel in caries progression and remineralization is critical. Moreover, roughness is an essential property of teeth, which influences the attachment of foreign materials to their surfaces [1]. Surface roughness governs the quality, color, buildup of plaque and performance of different surfaces and structures in the oral cavity [2].

The limit of surface roughness above which the bacteria would adhere is debatable [3]. The most commonly reported limit of surface roughness (Ra) is below 0.2 μm for adherence of dental biofilm and increase of roughness above this value lead to accumulation of bacteria [3]. Some remineralizing agents such as Remin Pro and MI Paste Plus decrease the surface roughness after bleaching [4].

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) [1]. 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 protects the enamel [5]. Remin Pro is another type of remineralizing agent, which contains calcium, phosphate, fluoride, and Xylitol [5]. Remin Pro contains hydroxylapatite particles that are deposited on the bleached enamel surface and increase the microhardness of teeth [4].

To our knowledge, limited studies have compared the influence of remineralizing agents on roughness of sound and demineralized enamel of primary teeth and no prior investigation has 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 primary teeth *in vitro*. The tested null hypothesis was that there is no difference between surface roughness of sound and demineralized enamel of primary teeth after application of different remineralizing agents.

Materials and Methods

The Ethical Committee approved (IR 0194) this investigation. Forty-eight primary 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, stored in 0.1% thymol, and used in this investigation. The teeth were prepared using a low speed water-cooled diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA) underwater and crowns were embedded horizontally with the buccal surface flat in partially set self-cure acrylic resin (Duralay; Reliance Dental C., Worth, IL, USA) in cylindrical plastic molds exposing approximately 7 × 7 mm enamel windows. Each buccal surface was divided into two sections/zones using a low speed water-cooled diamond saw. The specimens were randomly assigned into eight groups of 12 samples each. The power sample was calculated at a level of significance 0.05 and estimated standard deviation = 1 with power = 0.9583, 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) [3]. 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 [1]. Each sound and demineralized enamel group was placed in glass containers containing 50 ml artificial saliva (Pickering Laboratories, Inc., Mountain View, California, USA) at 37°C and pH 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.

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 1: *Distribution of different groups according to surface treatment of primary teeth enamel and remineralizing agents.*

Surfaces were kept wet with re-application every 15 minutes for a 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 at 37°C before the readings of surface roughness at time 2 (T2) were recorded similar to initial readings in the same zone assigned to each group. Specimens were stored in artificial saliva at 37°C 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 the previous application using a small brush for seven and a 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 at 37°C before the reading of surface roughness (T3) was recorded similar to the initial readings in the same zone assigned to each group. The specimens in the positive and negative control groups were kept in the artificial saliva at 37°C for the same time similar to the experimental groups.

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). Surface roughness is expresses, as an absolute value, the difference in height of each point compared to the arithmetical mean of the surface. It is defined as the shorter frequency of real surfaces relative to the troughs, therefore lower than the reference plane has a negative value (minus). Three-way ANOVA was used to test the interaction between times, types of enamel, and remineralizing agents. Then, within time and enamel, comparison between remineralizing agents was completed using one way ANOVA. This followed by using Tukey's multiple comparison test. All statistical analyses were set with a significance level of **α (**alpha) = 0.05. The statistical analysis was carried out with SPSS V21.0 (SPSS Inc., Chicago, Ill, USA).

Results

The surface roughness {Sa = Arithmetic mean height}, {Sp = Maximum peak height}, and {Sv = Maximum valley depth} of the enamel specimens were analyzed. The mean and standard deviation of the surface roughness "Sa", "Sp", and "Sv" of enamel as well as comparison and the statistical significance for sound and demineralized enamel following application of remineralizing agents and control at the three tested times is presented in table 2-4.

Time	Enamel	Remineralizing Agent	Mean	Std. Deviation	ANOVA (*p* value)	Tukey's Multiple Comparison Test			
						Control	Remin Pro	MI Paste	MI Paste Plus
T1***	Sound	Control	0.269	0.144	0.0001*	1			
		Remin Pro	3.3192	2.084		0.055	1		
		MI Paste	-3.399	1.158		0.002*	0.001*	1	
		MI Paste Plus	0.312	0.063		0.898	0.058	0.002*	1
	Demin**	Control	3.602	1.877	0.0001*	1			
		Remin Pro	-9.467	5.303		0.005*	1		
		MI Paste	0.466	0.194		0.033*	0.022*	1	
		MI Paste Plus	5.080	2.359		0.640	0.002*	0.018*	1
T2***	Sound	Control	-5.464	2.900	0.0001*	1			
		Remin Pro	0.358	0.110		0.016*	1		
		MI Paste	5.953	3.697		0.003*	0.088	1	
		MI Paste Plus	-8.972	5.743		0.628	0.071	0.008*	1
	Demin**	Control	0.344	0.083	0.0001*	1			
		Remin Pro	6.390	3.740		0.038*	1		
		MI Paste	-4.978	2.205		0.007*	0.001*	1	
		MI Paste Plus	0.403	0.279		0.958	0.040*	0.007*	1
T3***	Sound	Control	5.692	4.046	0.0001*	1			
		Remin Pro	-10.419	5.435		0.001*	1		
		MI Paste	0.315	0.075		0.078	0.017*	1	
		MI Paste Plus	6.944	4.808		0.960	0.001*	0.069	1
	Demin**	Control	-7.700	5.880	0.0001*	1			
		Remin Pro	0.306	0.106		0.072	1		
		MI Paste	4.198	3.401		0.011*	0.125	1	
		MI Paste Plus	-3.438	0.650		0.383	0.0001*	0.009*	1

Table 2: *Comparison and significance of "Sa" of sound and demineralized enamel following application of remineralizing agents and control at the three tested times.*

**: Significant.*

***: Demineralized.*

****: Time 1, 2, or 3.*

Time	Enamel	Remin eralizing Agent	Mean	Std. Deviation	ANOVA (p value)	Tukey's Multiple Comparison Test			
						Control	Remin Pro	MI Paste	MI Paste Plus
T1***	Sound	Control	0.267	0.141	0.0001*	1			
		Remin Pro	3.055	1.612		0.029*	1		
		MI Paste	-3.458	1.366		0.004*	0.0001*	1	
		MI Paste Plus	0.3140	0.064		0.874	0.032*	0.004*	1
	Demin**	Control	3.569	1.640	0.0001*	1			
		Remin Pro	-8.507	5.542		0.009*	1		
		MI Paste	0.325	0.1496		0.017*	0.041*	1	
		MI Paste Plus	4.020	3.414		0.991	0.006*	0.148	1
T2***	Sound	Control	-6.536	5.009	0.0001*	1			
		Remin Pro	0.388	0.107		0.068	1		
		MI Paste	6.939	6.070		0.009*	0.145	1	
		MI Paste Plus	-7.370	5.610		0.993	0.068	0.008*	1
	Demin**	Control	0.327	0.060	0.0001*	1			
		Remin Pro	4.381	4.107		0.191	1		
		MI Paste	-6.193	2.921		0.010*	0.003*	1	
		MI Paste Plus	0.382	0.273		0.959	0.198	0.010*	1
T3***	Sound	Control	6.051	5.016	0.0001*	1			
		Remin Pro	-5.287	3.021		0.006*	1		
		MI Paste	0.290	0.0765		0.124	0.023*	1	
		MI Paste Plus	1.940	1.042		0.305	0.005*	0.041*	1
	Demin**	Control	-5.386	3.052	0.0001*	1			
		Remin Pro	0.318	0.065		0.022*	1		
		MI Paste	2.685	1.830		0.003*	0.137	1	
		MI Paste Plus	-4.290	2.700		0.919	0.061	0.008*	1

Table 3: *Comparison and significance of "Sp" of sound and demineralized enamel following application of remineralizing agents and control at the three tested times.*

**: Significant.*

***: Demineralized.*

****: Time 1, 2, or 3.*

Time	Enamel	Remineralizing Agent	Mean	Std. Deviation	ANOVA (p value)	Tukey's Multiple Comparison Test			
						Control	Remin Pro	MI Paste	MI Paste Plus
T1***	Sound	Control	0.264	0.1384	0.0001*	1			
		Remin Pro	2.982	1.534		0.008*	1		
		MI Paste	-3.140	1.203		0.027*	0.0001*	1	
		MI Paste Plus	0.313	0.067		0.003*	0.029*	0.003*	1
	Demin**	Control	0.150	2.310	0.0001*	1			
		Remin Pro	3.819	1.866		0.014)	1		
		MI Paste	-8.150	6.054		0.023*	0.063	1	
		MI Paste Plus	0.413	0.310		0.957	0.017*	0.106	1
T2***	Sound	Control	3.213	2.322	0.0001*	1			
		Remin Pro	-4.168	1.492		0.003*	1		
		MI Paste	0.354	0.056		0.0001*	0.058	1	
		MI Paste Plus	3.602	2.243		0.439	0.044*	0.008*	1
	Demin**	Control	-7.721	5.182	0.0001*	1			
		Remin Pro	0.210	0.078		0.016*	1		
		MI Paste	2.704	1.1963		0.062	0.015*	1	
		MI Paste Plus	-5.746	4.268		0.942	0.016*	0.060	1
T3***	Sound	Control	0.358	0.246	0.0001*	1			
		Remin Pro	2.229	1.567		0.001*	1		
		MI Paste	-2.721	1.198		0.100	0.006*	1	
		MI Paste Plus	.299	0.081		0.932	0.027*	0.290	1
	Demin**	Control	3.134	3.417	0.0001*	1			
		Remin Pro	0.285	0.064		0.068	1		
		MI Paste	2.186	1.678		0.020*	0.020*	1	
		MI Paste Plus	-2.656	0.745		0.420	0.129	0.002*	1

Table 4: *Comparison and significance of "Sv" of sound and demineralized enamel following application of remineralizing agents and control at the three tested times.*
** Significant.*
***: Demineralized.*
****: Time 1, 2, or 3.*

ANOVA showed significant differences ($p < 0.0001$) of surface roughness "Sa" for the sound and demineralized enamel at each tested time (T1, T2, and T3) before and after application of different remineralizing agents and control (Table 2). The highest surface roughness "Sa" (mean ± SD) recorded for sound enamel were 3.319 ± 2.084, 5.953 ± 3.697, and 6.944 ± 4.808 for Remin Pro, MI Paste, and MI Paste Plus at T1, T2 and T3 respectively. The highest surface roughness "Sa" recorded for demineralized enamel were 5.080 ± 2.359, 6.390 ± 3.740, and 4.198 ± 3.401 for MI Paste Plus, Remin Pro, and MI Paste at T1, T2, and T3 respectively. Table 2 shows comparison between different remineralizing agents of sound and demineralized enamel and the statistical significance at T1, T2, and T3. For sound enamel of primary teeth at T2, a significant difference between the control and Remin Pro ($p < 0.016$) and MI Paste ($p < 0.003$) as well as between the control and Remin Pro ($p < 0.038$) and MI Paste ($p < 0.007$) for the demineralized enamel (Table 2). Sa for sound enamel at T3 after application of Remin Pro was statistically significantly different from control, MI Paste, and MI Paste Plus. Sa for demineralized enamel at T3 after application of MI Paste was statistically significantly different from control while MI Paste Plus was statistically significantly different from Remin Pro and MI Paste. The lowest surface roughness (Sa) at T3 was recorded for sound enamel after application of MI Paste and for demineralized enamel after application of Remin Pro for 8 hours.

Discussion

The null hypothesis of this *in vitro* study was rejected because there were differences between surface roughness of sound and demineralized enamel of primary 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 the samples were immersed into a glass container containing 50ml of demineralizing solution for a period of 72 hours at 37°C using an incubator. Another study used three hours as the period for demineralization in the pH cycling phase [4] to simulate the duration of demineralization that can occur in the oral cavity. However, it is relevant to emphasize that there are several dissimilarities between *in vivo* conditions and cycling models. The pH-cycling model does not entirely simulate the environments in the oral cavity where the pH oscillates repeatedly, and the levels reached depends upon practices of oral hygiene, usage of fluoride, eating habits of the individual, and the quality and composition of biofilm and saliva [4]. Thus, it would be beneficial if the remineralizing agents tested in the present study also would 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 [5]. Therefore, the application of remineralizing agents for 8 hours in the present study may not test long-term effect of the remineralizing agent on surface roughness. Minimal information is known regarding the surface roughness of enamel of primary teeth after application of different remineralizing agents. Furthermore, little is known about their influence on the short-term, which we 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 using the natural ability of saliva to remineralize artificial enamel caries [3]. 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 [2]. Another study reported that the presence of saliva could prevent the demineralizing effect of the bleaching agents on tooth enamel [6]. Thus, it would be interesting to use and test distilled water to show the behavior or effect on enamel. Sa and Ra are the most commonly reported parameters used to quantify surface roughness in the dental studies [7]. The present study showed significant difference between Sa of the sound and demineralized enamel of the control, Remin Pro, MI Paste, and MI Paste Plus after 8 hours application of the remineralizing agents and artificial saliva. In the present study, no attempt to change artificial saliva was performed as the previous study changed artificial saliva every 24 hours.

In the present study, the primary 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 surface of enamel have Retzius grooves, pits, and small defects which present a natural roughness as well as mineral deposits from the oral cavity [8]. The enamel surfaces used in this study were unground and only buccal surfaces were used. In addition, enamel in the middle third of the buccal surfaces were used to have a 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 [8]. 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 [9]. 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 influences the properties, such as a more mineralized surface (~9%), than inner enamel after eruption [9]. A study evaluated surface roughness of bleached enamel exposed to fluoride, MI Paste Plus, and Remin Pro found that the surface roughness was decreased compared to the initial enamel surface roughness and there was no difference between surface roughness of MI Paste Plus and Remin Pro groups [2]. In the present study, the highest surface roughness (Sa) was recorded at T2 for demineralized enamel treated with Remin Pro, which may indicate an increased roughness of the surface after 30 minutes of application compare to the negative control. In addition, the lowest surface roughness was recorded at T3 sound enamel, with no treatment/positive control but this was not significantly different from T1. In the present study comparison between sound and demineralized enamel after application of the remineralizing agents and control at T1 and T3 for primary teeth showed significant difference between the control, Remin Pro, MI Paste, and MI Paste Plus. A study evaluated topical application of CPP-ACP to bleached enamel demonstrated an improvement in surface hardness and a decrease in enamel surface roughness [10]. The 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 [11]. In contrast, a study in which bleached enamel surfaces were treated with MI Paste showed that surface roughness neither increased nor decreased [12]. Previous studies used different measurement methods to measure surface roughness of the enamel surface. In this study, non-contact optical profiler analysis was used to analyze the surface roughness (Sa, Sv, and Sp) in micrometer. 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 the biological specimen [13]. The greater the level of magnification during measurement of roughness, the lower Ra or Sa values measured for the same surface. Therefore, comparisons between surface roughness data of different studies have to be taken with thoughtfulness due to dissimilarities in settings and methods of surface analysis as well as tested surfaces. To our knowledge, no study reported human enamel three-dimensional roughness measured at a similar magnification has been published for comparisons to our study. 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. Surface roughness readings depend on the measurement method, thus the protocol for measurement of roughness is critical [14]. A contact profilometer with a stylus used for measurement of roughness moves in line, may induce misconception because of holes on the surface, and may injure enamel because of its contact with the surface [8,15]. Other instruments are used to measure roughness at a much higher resolution and over a larger area such as atomic force microscopes (AFM) and non-contact optical interferometers. In this investigation, the optical noncontact profilometer was used to measure surface roughness. Compared with a stylus profilometer, the optical interferometry noncontact profilometer is faster, nondestructive, and allows repeatability. A study concluded that the 3D optical profilometry technique was able to provide accurate qualitative and quantitative assessment of changes on the enamel surface after debonding [16].

It has been reported that addition of the fluoride to CPP-ACP could give a synergistic effect on enamel remineralization [17]. It has been suggested that the mechanism of remineralization of CPP-ACP comprises localization of ACP at the surface of the tooth to buffer free phosphate and calcium ions and these ions depress demineralization and promote remineralization [18]. 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 offers a higher quantity of available phosphate and calcium ions reservoir which makes it more effective in remineralization, and multiple scientific research studies provide that it can even remineralize enamel subsurface and early caries lesions [19]. The effect of the tested remineralizing agents on the enamel surface roughness may be related to the different composition of each agent, which is more than one ingredient and has its own manner in remineralization [20-22]. A study reported that using fluoride and hydroxylapatite in the case of Remin Pro increased enamel remineralization due to the creation of surface apatite coating the enamel, thus imitating the morphology, structure, composition and surface reactivity of the biological enamel hydroxylapatite [22]. The same study showed that, the CPPACPF led to significant reduction in surface roughness, which revealed that its remineralizing effect is greater than the fluoride gel, which may be due to the small size of the CPPACP nanocomplex [22]. It can enter the porosities of an enamel subsurface lesion and diffuse along its concentration gradient into the body of the lesion [23].

A high variation is seen in response to the protective agents for primary teeth, which was attributed to variation in porosity, lower content of phosphorous and calcium phosphate, and less organized microcrystals [24,25]. The degree of porosity in primary enamel explains the differences in demineralization and the tendency to dissolution in primary enamel compared with permanent teeth [26,27]. 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) [27]. How large impact the differences of the degree of porosity in the enamel have in demineralization *in vivo* is not yet known [28]. In addition, the chemical content and mineralization of enamel are known to vary between different teeth [29]. In the present study, demineralization was standardized for 72 hours. But it may not produce the same demineralization as the 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 [30].

In this study, we used continuous 30 min and 7.5 hours application of the remineralizing agent. This is experimental *in vitro* study, which test short and long-term application of three remineralizing agents that is why we used the method of continuous 30 minutes and 7.5 hours application of the remineralizing agents. The reason we used these two periods for the remineralizing agents is the reported time of application of the other remineralizing agents in different studies, which reported 40 minutes, 1.5 hours daily, and 24 hours [31-33]. In addition, a study used remineralization with artificial saliva for 21 hours for five consecutive days [34]. We used 30 minutes and 7.5 hours application of the remineralizing agent which is within the range of aforementioned studies. The clinical relevance of this study focus on the importance that morphological changes in the topography of enamel surfaces, especially if related to enamel roughness, are of considerable clinical importance. The quantitative evaluation method used herein enables comprehensive understanding of the effects of remineralizing agents on enamel of primary teeth.

The results of this research should consider the limitations, which include 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 [35]. Surface roughness *in vitro* may be dissimilar when compared to the dynamic conditions in the oral cavity *in vivo* and therefore, direct extrapolations to clinical conditions must be exercised with caution. However, in this *in vitro* study, standardization of experimental conditions was the advantage and the results demonstrated a clear correlation between surface roughness of enamel of primary teeth and application of the remineralizing agents and artificial saliva. 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.

Conclusion

The remineralizing agents tested in this study and artificial saliva showed significant differences on the surface roughness "Sa" for the sound and demineralized enamel of primary teeth at each tested time (T1, T2, and T3). MI Paste Plus showed the highest Sa for sound enamel and MI Paste for demineralized enamel after 8 hours of application of the remineralizing agent. Sa for sound enamel after application of Remin Pro was statistically significantly different than control, MI Paste, and MI Paste Plus after 8 hours of application of the remineralizing agent. Sa for demineralized enamel after application of MI Paste was statistically significantly different than control. While MI Paste Plus was statistically significantly different from Remin Pro and MI Paste after 8 hours of application of the remineralizing agent. The lowest surface roughness (Sa) was recorded for sound enamel after application of MI Paste and for demineralized enamel after application of Remin Pro for 8 hours.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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