

Pediatric dentistry and orthodontics Issue (Pediatric Dentistry, Orthodontics)

1-1-2023

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How to Cite This Article

Alattar, Wesam; Mostafa, Mohammed; and Hashem, Sara (2023) "The Effect of Remin Pro and MI Paste Plus on Induced Demineralized Enamel in Primary Molars," *Al-Azhar Journal of Dentistry*: Vol. 10: Iss. 1, Article 14.

DOI: <https://doi.org/10.58675/2974-4164.1470>

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The Effect of Remin Pro and MI Paste Plus on Induced Demineralized Enamel in Primary Molars

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Codex : 3-07/23.01

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<http://adjg.journals.ekb.eg>

<https://doi.org/10.58675/2974-4164.1470>

Pediatric Dentistry & Orthodontics
(Pediatric Dentistry, Orthodontics)

ABSTRACT

Purpose: To evaluate the remineralization potential of (Remin pro) nano hydroxy apatite (in comparison with MI paste plus, (Casein PhosphoPeptide-Amorphous Calcium Phosphate Fluoride) on demineralized enamel in human primary teeth. **Materials and Methods:** 44 primary extracted or exfoliated human primary molars teeth were equally divided into 2 groups (Group A, Group B). (Group A) including 22 primary teeth were treated with professional toothpaste (MI past plus). (Group B) including 22 primary teeth were treated with professional toothpaste (Remin Pro). Scanning electron microscope (SEM) readings and energy dispersive X-ray (EDX) analysis were carried at premineralization. The samples were subjected to the test agents after inducing white spot lesions. The readings were repeated demineralization and postremineralization. **Results:** SEM evaluation showed favourable surface changes in all the two study groups after remineralization therapy. EDX readings showed no significant differences between MI Paste Plus and Remin Pro ($P < 0.005$) in remineralization potential after 14 days. **Conclusion:** Use of MI Paste Plus and Remin Pro have a remineralizing effect on Induced demineralized enamel in primary molars.

INTRODUCTION

KEYWORDS

Enamel remineralization,
MI Paste Plus, Remin Pro.

Enamel demineralization is common in children and teenagers who have poor dental hygiene. The first clinical sign of enamel caries is a white spot lesion, or initial caries lesion, which is characterized

- Paper extracted from Master thesis titled “The Effect of Remin Pro and MI Paste Plus on Induced Demineralized Enamel in Primary Molars “
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by sub-surface enamel porosity from carious demineralization that appears on a smooth surface as a milky white opacity. These lesions are demineralized surfaces that are limited to enamel and are non-cavitated. They have a greater porous subsurface than sound enamel. White spot lesion is the earliest stage in the caries procedure and can be detained or remineralized at this stage ⁽¹⁾.

Noninvasive or minimally invasive treatments for these lesions are essential to avoid excessive tooth destruction caused by caries progression, and also minimize treatment time and cost. In addition, anxiety toward dental drilling is a severe problem in dental practice and leads to avoidance behavior, particularly in children. Routine operative treatment is challenging to perform and may require special behavior management ⁽²⁾.

For the treatment of white spot lesions, the most effective treatment is dental remineralization therapy. For enamel caries, fluoride is the most common remineralizing agent, in a number of studies ⁽³⁻⁴⁾, its benefits have been shown. Fluoride, when combined with calcium and phosphate ions, existing enamel crystals on the surface. Fluoride not only replaces minerals lost from the tooth structure, but is also significantly less dissolvable than the original hydroxyapatite carbonate ⁽⁵⁾.

Latterly, for the prevention and treatment of enamel caries, products containing calcium and phosphate ions have been developed and advocated. Remin Pro is composed of three effective compounds, including hydroxyapatite, fluoride, and xylitol, to protect against demineralization and enamel abrasion. The hydroxyapatite contained in this composition fills the surface enamel lesions and thus creates a smooth surface. Its fluoride content is 1450 ppm of sodium fluoride, and its xylitol content decreases the destructive impacts of bacteria and lactic acid metabolic effects, which are all effective in remineralization and strengthening the tooth enamel ⁽⁵⁾.

Since the 1980, when a topical anti-cariogenic activity of cheese was revealed, dairy products have been known to influence caries (milk, milk concentrate and cheeses). The protection effect was a direct chemical action of the concentration of phosphoprotein and calcium phosphate. Amorphous calcium phosphate nanoclusters are formed when casein phosphopeptide binds calcium and phosphate ions. Within the subsurface lesion concentration, these casein phosphopeptides - stable amorphous nanoclusters of calcium phosphate (CPP-ACP) can maintain high calcium and phosphate ion gradients and ion pairs. As the ion concentration in the lesion fluid rises, hydroxyapatite or fluorapatite is formed through crystal formation, minimizing enamel demineralization, and promoting remineralization. This combination has been demonstrated to result in significant remineralization of enamel lesions when appropriate levels of calcium and phosphate ions are combined with fluoride ions (CPP-ACPF). It was shown to be incorporated into the body of the spot lesion fluoride in combination with CPP-ACP rather than confined to the outermost surface of the enamel. Fluoride ions diffuse deep into the lesion, together with calcium and phosphate ions, allowing for considerable crystal formation (remineralization) throughout the lesion's body ⁽⁶⁾.

Enamel of primary teeth is less mineralized, exhibits a greater diffusion coefficient, and consequently more susceptible to acid dissolution compared to enamel of permanent teeth. Early childhood caries which affects the primary dentition frequently manifests as white spot lesions, and aggressive preventive therapy for remineralization of these lesions is essential for their reversal.

Thus, the aim of the present study was to evaluate the remineralization potential of commercially available agents containing hydroxyapatite (HA), casein phosphopeptide-amorphous calcium phosphate fluoride (CPP-ACPF), on artificially induced white spot lesions in primary molars teeth.

MATERIAL AND METHODS

This study was performed on 44 anonymous human primary sound molar teeth extracted due to shedding. Ethical approval for the use of extracted human teeth was obtained in accordance with guidelines from Research Ethics Committee (REC) Faculty of Dental Medicine for Girls, Al-Azhar University (REC-PE- 19-07). Teeth were cleaned by brushing under running tap water to remove organic debris and examined using a magnifying lens for the absence of caries, cracks, or anomalies. If any they were discarded. Specimens were stored in a daily renewed artificial saliva⁽⁷⁾. At the end of the study the teeth were disposed in medical waste container.

Sample grouping

The 44 extracted anonymous human primary molars teeth were divided equally into 2 Groups; (GroupA, Group B) to evaluate remineralization potential :

- Group A: 22 Primary molars were treated with professional toothpaste (MI past plus).
- Group B: 22 Primary molars were treated with professional toothpaste (Remin Pro).
- This was done the application of testing agent section.



Figure (1a&b): (A) is MI Paste Plus, (B) is Remin pro.

Sample preparation

The coronal section of the teeth was separated from the roots at the dentin-enamel junction with the use of a diamond disc, in the presence of a water coolant, installed in a micro motor straight hand piece (NSK, Japan). The buccal surface of the specimens was facing upwards while being fixed in epoxy resin. Acid-resistant nail varnish was applied to each tooth specimen leaving only a small window in the centre of the buccal surface of enamel (2x2 mm) exposed to the demineralizing solution, resulting in a caries-like lesion⁽⁸⁾. Before any cariogenic challenge, the exposed window was standardised in all specimens. At the beginning, an elemental (Ca/P) study was performed, followed by a structural examination.

Demineralizing regimen

Each sample was placed in 12 mL freshly prepared demineralizing solution in a sterile test tube (0.2ppm fluoride, 2.2mM CaCl₂, 2.2mM NaH₂PO₄, 0.05mM lactic acid). To produce artificial carious lesions, the pH was raised to 4.5 with % NaOH and maintained at 37°C for 72 hours⁽⁹⁾. Deionized water was used to properly wash the samples, which were allowed to dry after lesion formation. The sample was subjected to scanning electron microscopic (SEM) evaluation to evaluate surface morphology. Estimation of calcium (Ca), phosphate, and fluoride (F) (weight percentage) content was done using energy dispersive X-ray (EDX) analysis.

Application of test agents

For 14 days, the samples in each group were treated once every 24 hours with the allocated remineralizing agent. With the use of a disposable cotton tip applicator, the test agents were applied to the tooth surface within the window region for three minutes. After that, the samples were rinsed with deionized water and placed in artificial saliva at room temperature. Every 24 hours, before the

immersion of samples that were newly treated following a remineralization period of 14 days, artificial saliva was changed. SEM-EDX was used to examine the surface features of the samples and estimate the mineral content (Ca, phosphate [P], and F).

RESULTS

Scanning electron microscope

Premineralization:

SEM images (Figures 2a-3a) showing focal holes and perikymata.

MI paste group

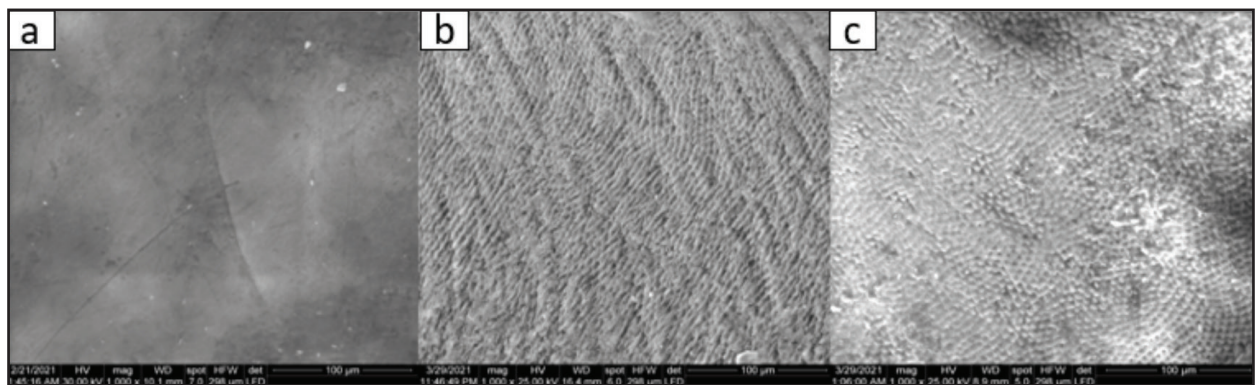


Figure (2): SEM of the enamel surface of the same sample in 3 stages: permineralization (a), demineralization (b), postmineralization (c) with MI paste plus (Orig.Mag. 1000x).

Remin pro group

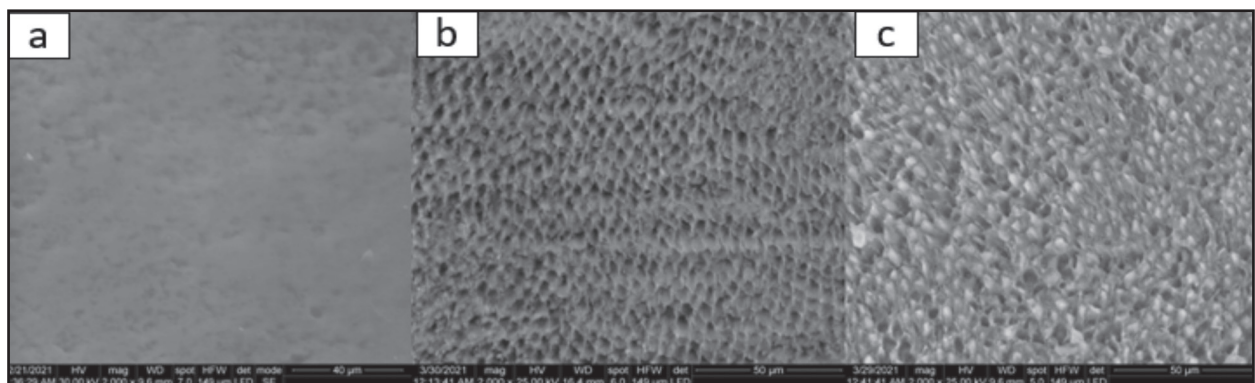


Figure (3): SEM of the enamel surface of the same sample in 3 stages: permineralization (a), demineralization (b), postmineralization (c) with Remin pro (Orig.Mag. 2000x).

Demineralization

SEM images (Figures 2b-3b) taken after 72 h of demineralization revealed loss of surface integrity. Alteration in prismatic structure of sound enamel, the enamel surfaces become porous and showed honeycomb appearance or showed varying degrees of areas of dissolution indicating demineralization.

Postmineralization

On observation SEM image (Figures 2c-3c), the samples demonstrated plugging of the porous defects with resultant decrease in the cavities and micropores.

Energy Dispersive X-ray Spectroscopy (EDX) calculation

Calcium concentration

By Comparison between group A and group B regarding calcium level pre mineralization, demineralization, and post mineralization (Table 1). There was no statistically significant difference between group A and group B regarding calcium level pre mineralization with a p-value = 0.292. The result also shows that the calcium level was significantly higher in group A than group B at demineralization with a p-value <0.001. Finally, no statistically significant difference found between group A and group B regarding calcium level post mineralization with a p-value = 0.079.

Phosphorus concentration

By comparison between group A and group B regarding phosphorus level pre mineralization, demineralization, and post mineralization (Table 2). The results show that there was a statistically significant difference between group A and group B regarding phosphorus level pre mineralization with a p-value=0.002. Also, the phosphorus level was found higher in group A than group B demineralization with a statistically significant difference between them with a p-value < 0.001. Finally, no statistically significant difference was found between group A and group B post mineralization with a p-value = 0.426.

Table (1) Comparison between group A and group B regarding Calcium level pre mineralization, demineralization and post mineralization.

Stages	Groups	Mean	SD	SE	95% CI for mean		Min.	Max.	Test value	P-value	Sig.
					Lower	Upper					
Pre-mineralization	Group A	46.43	2.27	0.48	45.42	47.43	42.56	50.44	-1.067	0.292	NS
	Group B	47.30	3.07	0.66	45.93	48.66	41.43	55.88			
Demineralization	Group A	33.70	1.49	0.32	33.04	34.37	28.89	35.75	4.056	0.000	HS
	Group B	26.87	7.76	1.66	23.42	30.31	12.1	42.7			
Post-mineralization	Group A	39.84	2.54	0.54	38.72	40.97	36.01	46.55	1.800	0.079	NS
	Group B	38.38	2.84	0.61	37.12	39.64	32.47	42.3			

P-value > 0.05: Nonsignificant; P-value < 0.05: Significant; P-value < 0.01: Highly significant •: Independent t-test

Table (2) Comparison between group A and group B regarding phosphorus level pre mineralization, demineralization, and post mineralization.

Stages	Groups	Mean	SD	SE	95% CI for mean		Min.	Max.	Test value	P-value	Sig.
					Lower	Upper					
Premineralization	Group A	19.54	0.59	0.12	19.28	19.80	18.6	21.21	3.344•	0.002	HS
	Group B	18.57	1.23	0.26	18.02	19.11	16.37	20.48			
Demineralization	Group A	13.90	1.45	0.31	13.26	14.55	8.82	16.31	4.188•	0.000	HS
	Group B	9.48	4.74	1.01	7.38	11.58	2.18	19.45			
Postmineralization	Group A	17.34	0.69	0.15	17.04	17.65	16.08	19.45	0.804•	0.426	NS
	Group B	17.03	1.71	0.36	16.27	17.78	13.8	19.86			

P-value > 0.05: Non significant; P-value < 0.05: Significant; P-value < 0.01: Highly significant •: Independent t-test

DISCUSSION

Different approaches can be used to assess in vitro demineralization and remineralization. The methods used in the present study were EDX-SEM. EDX analysis was used to demonstrate both Ca/P ratios and total weight percentages of calcium and phosphate following remineralization treatment, before and after demineralization of enamel in primary teeth. EDX analysis, used in conjunction with SEM, is a sensitive method of qualitative and quantitative compositional analysis. An electron beam that has been carefully focused (electron probe) is bombarded on the surface of a conducting sample. The wavelength and intensities of the characteristic X-ray emitted from the material and the intensities of secondary electrons and backscattered electrons are measured⁽¹⁰⁾. Specimens were maintained for simulation of clinical conditions in artificial saliva⁽¹¹⁾.

The two separate remineralizing agents containing fluoride were selected in this study (Remin Pro, MI Paste Plus) due to the importance of fluoride in enamel remineralization⁽¹²⁾.

Remin Pro can infill the porosities in the enamel and forms a protective layer on the surface of the tooth to prevent bacterial plaque adherence to the tooth surface. Moreover, xylitol contained in Remin Pro prevents caries by interfering with the metabolism action and the fermentation of sucrose reducing acid production by microorganisms⁽¹³⁾.

The use of CPP-ACP for the prevention and treatment of enamel caries has increased in recent years. CPP-ACP has been shown to stabilize calcium phosphate in dental plaque in the proximity of the teeth. In the presence of acid challenge, such as after each meal, CPP-ACP helps to preserve a supersaturated state of minerals with respect to dental enamel and thus restricts mineral loss and assists remineralization of enamel subsurface lesions. CPP-ACP and 900 ppm fluoride are the composition of MI Paste Plus. It was reported that the addition of fluoride into CPP-ACP enhances its

remineralization potential on initial enamel lesions in comparison with either sodium fluoride alone or CPP-ACP⁽¹⁴⁾.

The result of the current study showed that there was no significant difference in remineralization effect between MI paste and Remin pro. According to the findings of the research, on the reduction of enamel surface roughness, the effects of CPPACPF (MI paste plus) and Remin Pro were almost comparable. There was no significant difference in their remineralizing effect. These results may be due to the fact that both Remin Pro and MI Paste plus consist of more than one ingredient that plays a role in remineralization. Other studies⁽¹⁵⁻¹⁶⁾ results agree with these findings⁽¹³⁾. The mean surface hardness of MI Paste plus group specimens was lower compared to the sound enamel group. Contrary to this, in a previous study, surface hardness of the MI paste group was observed to be higher compared to the sound dental enamel group⁽¹⁷⁾.

MI Paste Plus is more efficacious in remineralizing the tooth structure than the Remin Pro⁽¹⁸⁾, which was different from results of the present study. Although high calcium concentrations have been previously demonstrated to be effective in dental remineralisation, high calcium concentration can lead to rapid absorption in the surface layers, and hence, reduced remineralization in deeper lesion layers⁽¹⁹⁾. MI Paste Plus contains casein phosphopeptide, which prevents rapid deposition and stabilizes calcium and phosphate compounds.⁽²⁰⁾ Because of the reduced amount of remineralization, MI Paste can be used as a fluoride additive but not as a fluoride alternative.

It is important to note that natural dental biofilm and saliva create an ideal Environments for maintaining the supersaturated condition of ions in the oral cavity during CPP-ACP therapy. The effect of these compounds on dental samples can only be attributed to their application duration due to the absence of these variables in vitro; this explains the disparity between in vivo and in vitro studies⁽²¹⁾.

CONCLUSION

Remineralizing products such as Remin Pro and MI Paste Plus have a remineralizing effect on induced demineralized enamel.

RECOMMENDATIONS

1. Further in vivo investigation studies are needed to examine the efficacy of the remineralizing agents in the highly challenging oral environment.
2. In order to acquire more conclusive results, further studies must be done on the formation and chemical structure of enamel crystals using advanced quantifying techniques and on the acid solubility resistance of these remineralized crystallites.

ACKNOWLEDGMENT

This paper results and research behind it would not have been possible to be completed without the support of Main Defence Chemical Laboratory, Cairo, Egypt.

Conflict of Interest

None declared .

Finding

No funding was received for this study.

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