EGYPTIAN
DENTAL JOURNAL
www.eda-egypt.org

VOL. 68, 4075:4084, OCTOBER, 2022
PRINT ISSN 0070-9484 • ONLINE ISSN 2090-2360

CONSERVATIVE DENTISTRY AND ENDODONTICS

Submit Date : 29-08-2022 • Accept Date : 19-10-2022 • Available online: 01-10-2022 • DOI : 10.21608/edj.2022.159370.2236

EFFECT OF (CPP-ACP) AND (CPP-ACP WITH FLUORIDE) ON CHEMICAL AND MORPHOLOGICAL ANALYSIS OF BOVINE TEETH FOLLOWING DEMINERALIZATION/ REMINERALIZATION CYCLES (AN IN-VITRO STUDY)

Shadwa Hatem Kabil[*], Mohamed Zayed[**] *and* Shaimaa Rohym[***]

ABSTRACT

Objective: To compare the remineralization outcome of CPP-ACP without or with fluoride "MI Paste" & "MI Paste Plus" on the re-mineralization of enamel caries-like lesions in bovine teeth.

Material and methods: 21 clean sound bovine teeth were collected and prepared. All samples were mounted in acrylic molds. Randomization was done to divide them into 3 groups (n= 7 teeth); (GPI: Control Group no treatment; were not exposed to demineralization nor remineralizing agents only exposed to synthetic saliva, GPII: MI Paste and GPIII: MI Paste Plus. Each group was examined by (SEM) with magnifying range 6000,12000x and EDX analysis were taken place before demineralization, after 72 hrs of demineralization and after 21 days of remineralization. Data were analyzed by using Independent t-test to compare between different groups, while comparison between control, demineralization and remineralization was performed by using One Way ANOVA test followed by Tukey`s Post Hoc test for multiple comparisons.

Results: Morphological changes of enamel surfaces of "MI Paste" and "MI Paste Plus" showed better appearance. For chemical analysis "MI Paste Plus" showed significantly better results than "MI Paste" with an increased amount of calcium & calcium/ Phosphate ratio.

Conclusion: Results emphasize the positive effect of both pastes in enamel remineralization. Also, addition of fluoride in "MI Paste Plus" had a synergistically effect with the CPP-ACP and provided better remineralization.

KEYWORDS: MI Paste, MI Paste Plus, reminerlization & demineralization cycles

* Assistant Professor, Restorative Dentistry, Faculty of Dentistry, The British University in Egypt, Cairo, Egypt
** Associate Professor, Conservative Dentistry, Faculty Of Dentistry, Sinai University Kantara Branch, Cairo, Egypt
*** Assistant Professor, Operative Dentistry, Al-Ahram Canadian University, Cairo, Egypt

INTRODUCTION

White Spot lesion (WSL) is the first clinical sign of enamel demineralization. The equilibrium between demineralization and remineralization process, are affected by pathological factors and protective factors that determine the fate of the lesion (1). If these demineralized lesions kept untreated, further progress of minerals loss might occur and breakdown until cavitation (2).

Recently, researches of cariology examined the effectiveness of the application of some remineralizing agents that depend on supersaturation of ions in the oral environment surrounding these lesions. Providing calcium and phosphate ions to the tooth to promote ions deposition into crystal voids in demineralized enamel, providing refill to the formed microspores and stop further mineral loss (3).

Non-fluoride (bioavailable calcium phosphate) remineralizing agents are now introduced to be the gold standard medical treatment for white spot lesions. Term of bioactive remineralizing agents includes tricalcium phosphate, amorphous calcium phosphate (ACP) and casein phosphopeptide (CPP-ACP) (4).

Casein phosphopeptides (CPP) are products of milk protein casein. Their anti-cariogenic properties are due to the presence of casein, calcium and phosphate, which are responsible for depressing acid dissolution (3,5). MI Paste™ and MI Paste Plus™ are preventive treatment products, were advertised to help prevent demineralization, and improve remineralization (6,7).

The purpose of this in vitro study was to evaluate and compare the effects of remineralizing agents (MI Paste™ and MI Paste Plus™) on the surface topography and chemical analysis of sound bovine enamel following demineralization and remineralization cycles as there is a limited data regarding this issue. The null hypothesis of this study was there would be no significant difference between "CPP-ACP" with or without fluoride in the remineralization of enamel in bovine teeth.

MATERIALS

Two commercially available reminerlizing agents were used for this study; MI Paste™ (GC, Europe N.V.) and MI Paste Plus™ (GC, Europe N.V.). These professional use products contain RECALDENT ™ (CPP-ACP), a special milk derived protein with source of bio-calcium and phosphate (and fluoride in MI Paste Plus) to the tooth surfaces.

Sample size calculation:

Sample size calculated depending on a previous study as reference (8). According to this study, the minimally accepted sample size was 5 per group, when the response within each subject group was normally distributed with standard deviation 0.18, the estimated mean difference was 0.33, when the power was 80 % & type I error probability was 0.05. Total sample size per group increased to 7 to compensate 15 % drop out.

METHODOLOGY

a) Specimen preparation

Twenty-one clean sound bovine incisors were collected and prepared. A 10x7-mm tape was placed on the labial surface of each tooth after cutting the root and removing the pulp, and nail varnish was applied in the remaining areas of the surface. After the nail varnish was dried, the bovine incisors were embedded in acrylic resin (Ortho-Jet, Lang Dental, USA) then the tape removed to form a window (Figure 1). All specimens were immersed in distilled water in a sealed container (9). Computer Randomization of specimens were done to divide them into three groups (n= 7 teeth); (GPI: Control Group no treatment; were not exposed to demineralization nor remineralizing agents only exposed to synthetic saliva, GPII: MI paste and

GPIII: MI Paste Plus. Each group was examined for scanning electron microscope observation (SEM) with magnifying range 6000x to 12000x. And Energy Dispersive X-ray spectroscopy (EDX) (QUANTA FEG 250) analysis were taken place before demineralization cycles, after 72 hours of demineralization cycles and after 21 days of remineralization cycles. This experimental in-vitro study had received approval from the research ethics committee of Faculty of dentistry, The British University in Egypt. (Research approval Number: 22-016)

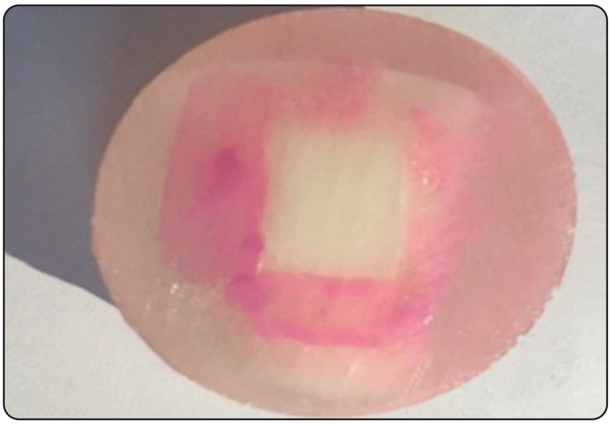

Fig. (1): Bovine tooth embedded in acrylic resin and window formation by tape

b) Demineralization & Remineralizing Cycles:

Each specimen was immersed in 10 mL of demineralizing solution (Composed of 2.2 mM calcium chloride, 2.2 mM potassium dihydrogen phosphate, 0.05 M acetic acid, and 1 M potassium hydroxide (KOH) to maintain a pH of 4.4) for 72 hours to create artificial caries (10). Then, they were rinsed with distilled water and stored in artificial saliva (Artificial saliva was prepared by mixing 500 ml distilled water, 20 g potassium chloride, 0.843 g sodium chloride, 0.051 g magnesium chloride, carboxymethyl cellulose, 20 ml tricalcium phosphate, and 0.05 M sodium hydroxide to maintain a pH of 6.8) to simulate the oral cavity conditions (11). Every 12 hours, the demineralizing solution was renewed to prevent depletion of solution.

Remineralizing pastes were used according to manufacturer's instructions: a single application of approximately 0.5 mm layer for 3 minutes (12, 13); twice daily with artificial saliva. Rinsing between remineralizing paste application was done, followed by rinsing for 21 days with fresh daily synthetic saliva (14).

c) Statistical Analysis:

Statistical analysis was performed with SPSS 16 ® (Statistical Package for Scientific Studies), Graph pad prism & windows excel and presented in two tables and two graphs. Exploration of the given data was performed using Shapiro-Wilk test and Kolmogorov-Smirnov test for normality which revealed that all data originated from normal distribution (parametric data) resembling normal Bell curve. Accordingly, comparison between different groups was performed by using Independent t-test, while comparison between control, demineralization and remineralization was performed by using One Way ANOVA test followed by Tukey`s Post Hoc test for multiple comparisons.

RESULTS

A. Morphological Changes:

Teeth were examined at magnifications of 6000x & 12000 x with SEM, enamel surfaces after de-minerization cycle showed type 1 etching pattern; partial loss of the central prism core and retention of the prism periphery (Figure 2). Enamel surfaces of demineralized enamel followed by MI Paste and synthetic saliva rinsing showed layer of disaggregated nanoclusters with a globular structure that decreased the obvious porosities (Figure 3). While enamel surface of demineralized enamel followed by MI Paste Plus showed relatively more dense surface coatings with more spread focal areas with no obvious porosities. (Figure 4)

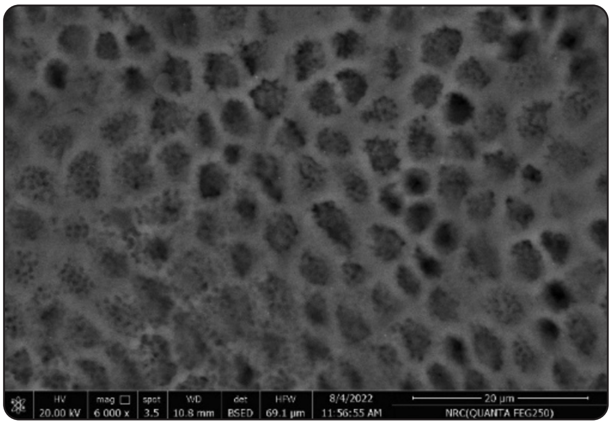

Fig. (2): After 72 hours of demineralization cycles

B. Chemical Analysis:

I. Evaluation of Calcium, Phosphorous and Calcium / Phosphorous ratio between MI paste and MI paste plus groups:

- Calcium:

Comparison between both groups revealed insignificant difference between them as P >0.05 regarding control as MI paste (21.23 ± 0.93) was insignificantly lower than MI Paste plus (21.57± 0.85). And in demineralization MI Paste (11.86 ± 2.27) was insignificantly lower than MI Paste Plus (12.17± 2.57), while in remineralization there was a significant difference between them as P < 0.05

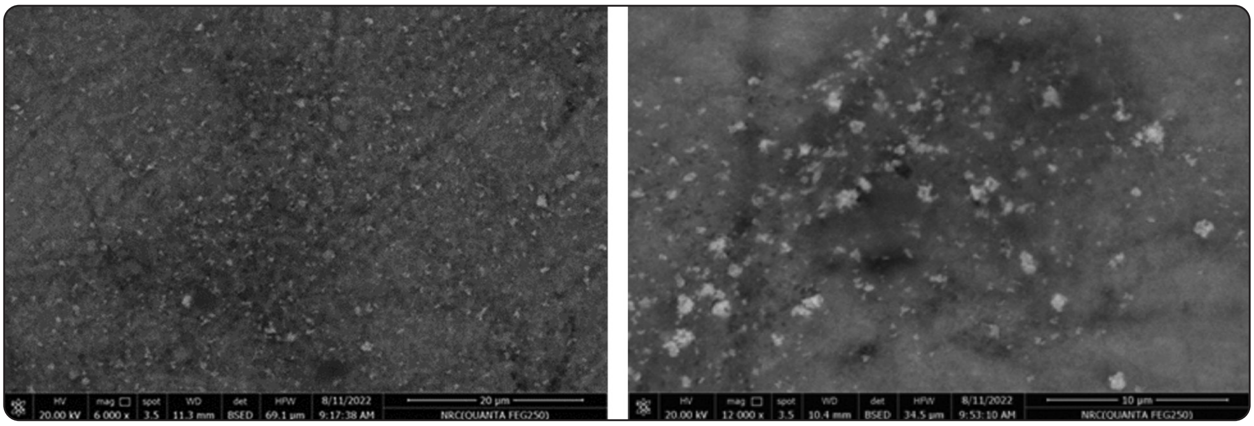

Fig. (3): After remineralization by MI paste (6000x and12000x)

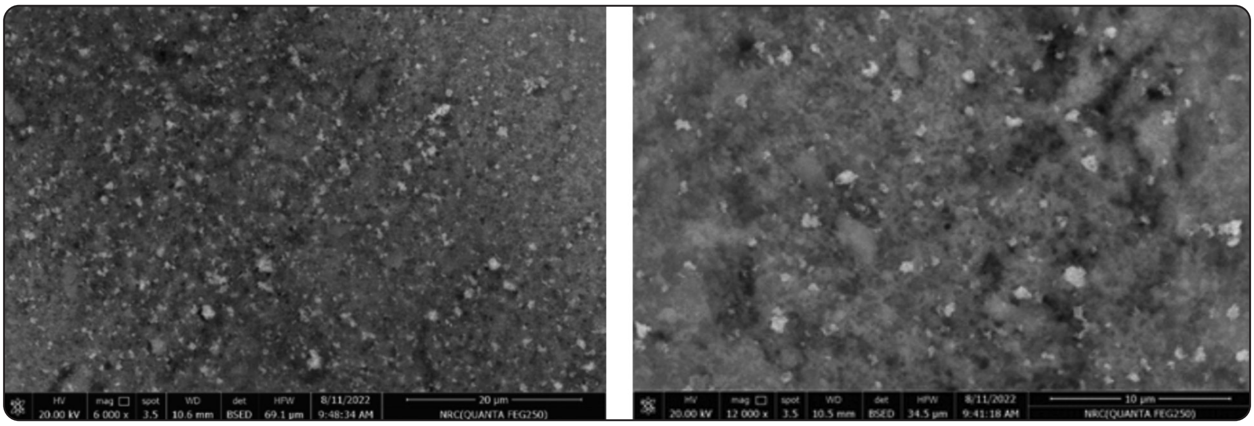

Fig. (4): After remineralization by MI paste plus (6000x and 12000x) as MI Paste (17.23 ± 0.66) was significantly lower than MI Paste Plus (21.14± 0.47), as presented in table (1) and figure (5).

- Phosphorous:

Comparison between both groups revealed insignificant difference between them as P >0.05 regarding control as MI Paste (12.54 ± 0.15) was insignificantly lower than MI Paste Plus (21.57± 0.85). And in demineralization MI Paste (10.09 ± 1.71) was insignificantly lower than MI Paste Plus (10.57± 1.4). While in remineralization there was a significant difference between them as P < 0.05 as MI Paste (11.13 ± 0.59) was significantly lower than MI Paste Plus (12.28 ± 0.31), as presented in table (1) and figure (5).

- Calcium / Phosphorous ratio:

Comparison between both groups revealed insignificant difference between them as P >0.05 regarding control as MI Paste (1.69 ± 0.06) was significantly lower than MI Paste Plus was (1.70± 0.03). And in demineralization MI Paste (1.17 ± 0.06) was significantly higher than MI Paste Plus was (1.15± 0.08). While in remineralization there was a significant difference between them as P < 0.05 as MI paste (1.54 ± 0.08) was significantly lower than MI paste plus (1.72 ± 0.07), as presented in table (1) and figure (5).

II. Evaluation of Calcium, Phosphorous and Calcium / Phosphorous ratio between Control, Demineralization, and Remineralization groups:

- Calcium:

In MI Paste, there was a significant difference between control (the highest), demineralization (the lowest) and remineralization. While in MI Paste Plus demineralization was significantly the lowest, whilst there was insignificant difference between control and remineralization as presented in table (1) and figure (5).

TABLE (1) : Mean and Standard Deviation of Calcium, Phosphorous and Calcium/Phosphorous ratio in MI paste and MI paste plus regarding Control, Demineralization , Remineralization Groups:

		MI Paste		MI Paste Plus		P value
		M	SD	M	SD	
Calcium	Control	21.23 a	0.93	21.57 a	0.85	0.48
	Demineralization	11.86 b	2.27	12.17 b	2.57	0.81
	Remineralization	17.23 c	0.66	21.14 a	0.47	<0.0001*
	P value	<0.0001*		<0.0001*		
Phosphorous	Control	12.54 a	0.15	12.66 a	0.18	0.21
	Demineralization	10.09 b	1.71	10.57 b	1.4	0.56
	Remineralization	11.13 ab	0.59	12.28 a	0.31	0.0006*
	P value	0.001*		0.004*		
Calcium / Phosphorous ratio	Control	1.69 a	0.06	1.70 a	0.03	0.71
	Demineralization	1.17 b	0.32	1.15 b	0.08	0.87
	Remineralization	1.54 a	0.08	1.72 a	0.07	0.0008*
	P value	0.0003*		<0.0001*		

M: mean ***SD: standard deviation*** ****Significant difference as P <0.05***

Means with the same superscript letters were insignificantly different a P>0.05

Means with different superscript letters were significantly different a P<0.05

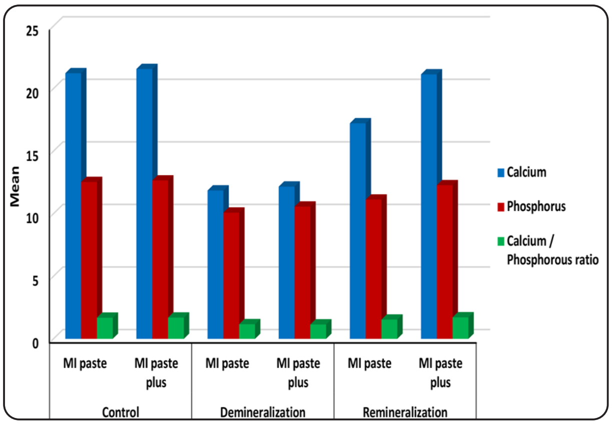

Fig. (5): Bar Chart showing Mean of Calcium, Phosphorous and Calcium/Phosphorous ratio in MI paste and MI paste plus regarding Control, Demineralization, Remineralization Groups.

- Phosphorous:

In MI Paste, control was significantly the highest, while demineralization was significantly the lowest and remineralization revealed insignificant difference with control and demineralization. In MI Paste Plus demineralization was significantly the lowest. At the same time there was insignificant difference between control and remineralization as presented in table (1) and figure (5).

- Calcium / Phosphorous ratio:

In both MI paste and MI paste plus demineralization was significantly the lowest, while there was insignificant difference between control and remineralization as presented in table (1) and figure (5).

III. Evaluation of Calcium, Phosphorous and Calcium / Phosphorous ratio in Control- Demineralization Changes and in Demineralization-Remineralization Changes:

- Control – Demineralization Changes: Calcium in MI Paste decreased with (-9.37 ± 0.37) and MI Paste Plus decreased with (-9.4 ± 0.71), while phosphorous MI Paste decreased with (-2.45 ± 0.54) and MI Paste Plus decreased with (-2.09 ± 0.52). In calcium / phosphorous ratio MI Paste decreased with (-0.52 ± 0.26) and MI Paste Plus decreased with (-0.55 ± 0.05). Comparison between both groups was performed by using independent t-test revealed insignificant difference between them as P<0.05, as presented in table (2) and figure (6).

Demineralization-Reminerlization Changes:

Calcium in MI Paste increased with (5.37± 0.62) and MI Paste Plus decreased with (8.97 ± 0.79),

TABLE (2): Mean difference and standard deviation of calcium. Phosphorous and calcium/phosphorous ratio changes in MI paste and MI paste plus regarding Control - Demineralization and Demineralization – Remineralization Groups:

		Calcium		Phosphorus		Calcium /phosphorous ratio	
		MD	SD	MD	SD	MD	SD
Control - Demineralized	**MI paste**	-9.37	0.37	-2.45	0.54	-0.52	0.26
	MI paste plus	-9.4	0.71	-2.09	0.52	-0.55	0.05
	P value	0.92		0.22		0.76	
Demineralized - Remineralized	**MI paste**	5.37	0.62	1.04	0.12	0.37	0.14
	MI paste plus	8.97	0.79	1.71	0.11	0.57	0.01
	P value	<0.0001*		<0.0001*		0.002*	

MD: mean difference *SD: standard deviation* **Significant difference as P <0.05*

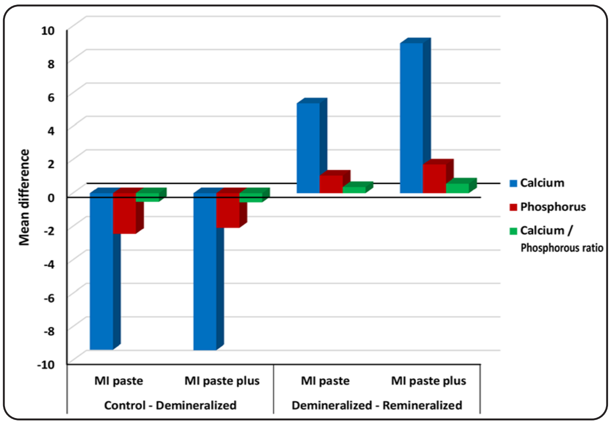

Fig. (6): Bar Chart showing Mean difference of Calcium, Phosphorous and Calcium/Phosphorous ratio changes in MI paste and MI paste plus regarding Control - Demineralization and Demineralization – Remineralization.

whilst phosphorous MI Paste increased with (1.04 ± 0.12) and MI Paste Plus increased with (1.71± 0.52). In calcium / phosphorous ratio MI Paste increased with (0.37 ± 0.14) and MI Paste Plus decreased with (0.57 ± 0.01). Comparison between both groups was performed by using Independent t-test revealed significant difference between them as $P < 0.05$ (MI Paste Plus was significantly higher than MI Paste in calcium, phosphorous and calcium / phosphorous ratio), as presented in table (2) and figure (6).

DISCUSSION

The null hypothesis of our study was rejected since "CPP-ACPF" showed better re-mineralization potential than "CPP-ACP" solitary. In this study, we utilized qualitative and quantitative methods to assess remineralization of the artificial caries lesion in order to give more broad details. In order to relate between the quantitative results of the calcium and phosphate content in enamel and the microscopic appearance of enamel surface after demineralization /remineralization process.

Bovine teeth were assigned in this study because of the similarity in the chemical composition, hardness and the affordability [15]. Human saliva plays vital role in enamel remineralization related to major inorganic components (e.g., calcium, phosphate, and fluoride) and organic components (e.g., glycoproteins). The time that oral environment changes, saliva will lose its protective effects due to changes in its composition [16]. Also difficulties in collection and time consuming limits the use of natural saliva in in vitro studies [16]. This reason illustrates the use of substitutive formulations (artificial saliva) in our study, to simulate the oral environment in remineralization studies [16].

Energy Dispersive X-ray spectroscopy (EDX) is considered as a quantitative micro-analytical technology. It provides adequate information about the chemical composition of the targeted area of a sample. Such technique is mainly depends on the emission of a characteristic X-ray through a filament toward the sample [17]. Specific elements are represented in a histogram plot on EDX detector with the number of counts against the X-ray energy [17]. In combination with SEM magnifies and scans the area of interest and detects micro-morphological changes [18]. It was categorized that SEM–EDX is a semi-quantitative approach for elemental analysis [19]. On the other side, it was considered that the utilization of electron probe microanalysis generating X-ray in quantifying enamel mineral changes is a highly problematic approach including non-uniform porosity that may cause density variations, an observed reduction of generated X-ray, and low interaction volumes for nonhomogeneous samples [20]. All these drawbacks justify using SEM–EDX in conjunction with another quantitative approach.

As demonstrated by scanning electron microscopy in our study, application of both protective agents enhanced creation of superficial mineral layer. The protective layer in MI Paste Plus was more evident with calcified deposits and concentrated along the porous defects when compared with MI paste. This was in accordance with many studies [21, 22, 23]. Both test groups showed significant remineralization after twenty-one days of treatment with “MI Paste or MI Paste Plus”. This is may be due to the mechanism of action in these bioactive materials. When such products placed on the tooth surface it interacts with hydrogen ions forming the same species of calcium hydrogen phosphate. Then it enters into the porous enamel under a diffusion gradient action to interact with and consume the water to reproduce enamel minerals, thus compensating subsurface mineral loss. [24,25] The efficacy of their technology in preventing demineralization and boosting re-mineralization of enamel in vivo [26,27]. Our study demonstrated that addition of fluoride to the paste “MI Paste Plus”, had accentuated better remineralization, this was due to the ability of “CPP” to connect fluoride as well as calcium and phosphate, forming calcium fluoride phosphate as soluble complexes [23]. These findings were supported by many studies [28, 29].

Confirming the Scanning Electron Microscope (SEM) findings of this study, the energy dispersive x-ray spectroscopy (EDXS) results also showed that both groups treated with MI Paste and “MI Paste Plus” significantly increased the mean calcium content, phosphorous content and Ca/P ratio when compared to their demineralized groups. It was found that the ability of “CPP” to stabilize nanoclusters of amorphous calcium phosphate, inhibit their growth into the critical size into phase transformations [30, 31]. This allow bioavailability of ions for diffusion through the lesions deficient in minerals .Demineralized crystals become re-mineralized though omission surface deposition in the shape of calculus [27]. The current results agreed with a study, who explained that the enhanced enamel re-mineralization was due to the presence of both inorganic calcium and phosphorus in high concentrations in the “CPP-ACP” [32].

The significance difference in in control group (no treatment) and demineralized group was due to significant loss of ions of Ca, Phosphate content due to low PH demineralization cycle [8].

Comparing MI Paste with the “MI Paste Plus”, there was a significant increase in the mean calcium content and calcium/ phosphorus ratio in “MI Paste Plus” than the “MI Paste” specimens. Also, insignificant difference was found between MI Paste Plus and Control group. The better results in MI Paste Plus could be due to the limitation of the amorphous calcium fluoride phosphate “ACFP” at the tooth surface by the casein phosphor-peptides (CPP). Co-localizing calcium, phosphate and fluoride ions and maintain supersaturation that suppress demineralization [33,34]. Findings in our study were in accordance with many studies.[8,23,31, 35]

On the contrary to our results, a study stated that the application of “MI Paste Plus” in patients with post-orthodontic white spot lesions did not improve these lesions [36]. It could be due to the dynamic change in salivary pH in patients that depend on various individual variations. Moreover, the dietary habits and amount of sucrose ingested that can decrease the salivary pH to a great extent. Therefore, this would hindered the effect of “MI Paste Plus” and prevent remineralization process. Furthermore, constant PH (6.8) of the artificial saliva in our study with constant remineralization time may enhanced the action of remineralizing agent.

This could be considered one of the limitations of the in vitro studies that might not accurately mimic in the oral cavity environment giving different results than in vivo studies. Other limitation was found that the formula of artificial saliva used in this study contains carboxymethyl cellulose. This component increases the viscosity of the artificial saliva solution, which may limit the mineral diffusion to the enamel substrate. Also, it can form complexes between calcium and phosphate ions, hindering such minerals in enamel remineralization. Selecting such a formula was done to avoid generating misleading results and providing some sort of accurate evaluation of tested materials.

CONCLUSION

The following can be concluded:

1. The addition of fluoride to "CPP-ACP" in the form of "CPP-ACPF" gave higher ranking remineralization results than "CPP-ACP" alone.
2. "CPP-ACP" [**MI Paste, GC**] and "CPP-ACPF" [**MI Paste Plus, GC**] were efficacious in the remineralization of enamel caries-like lesions.

Conflict of interest:

"The authors declare that they have no conflicts of interest."

REFERENCES

1. Taneja V, Nekkanti S, Gupta K, Hassija J. Remineralization potential of theobromine on artificial carious lesions. JISPCD. 2019;9(6):576-583.
2. Alaff A., Yassen A. A. & Hassanein O. E. Efectiveness of polyacrylic acid-bioactive glass air abrasion preconditioning with NovaMin remineralization on the microhardness of incipient enamel-like lesion. JCD.2019; 22, 548–553.
3. Reynolds EC. Calcium phosphate-based remineralization systems:scientific evidence? Aust Dent J. 2008;53(3): 268-73
4. Ismail, Amid I., Sohn, Woosung; Tellez, Marisol, Amaya, A.;Sen, A., Hasson, H, Pitts, N. B. The International Caries Detection and Assessment System (ICDAS): An integrated system for measuring dental caries. Commun. Dent. Oral Epidemiol. 2007;35, 170–178.
5. Manoharan V, Kumar RK, Sivanraj AK, Arumugam SB. Comparative evaluation of remineralization potential of casein phosphopeptide-amorphous calcium fluoride phosphate and novamin on artificially demineralized human enamel: An in vitro study. Contemp Clin Dent. 2018;9(1):58-63.
6. Laurence J. W. Contemporary technologies for remineralization therapies: A review International Dentistry SA 2009; 11(6):6-16.
7. Remineralizing effectiveness of MI Paste Plus- a clinical pilot study. Sarah Elizabeth Clark, University of Iowa (2011)
8. Hamdi, K., Hamama, H.H., Motawea, A. et al. Remineralization of early enamel lesions with a novel prepared tricalcium silicate paste. Sci Rep 12, 9926 (2022).
9. Ju-Lee Son, Yoon-Jeong Shin, Geon-Hee Jeong, Shin-Jae Choi, Seunghan Oh, Ji-Myung Bae. "Effect of fluoride varnishes on the surface hardness of bovine teeth under demineralization/remineralization cycling." (2020).
10. Kumar, V & Itthagarun, Anut & King, Nigel. (2008). The effect of casein phosphopeptide-amorphous calcium phosphate on remineralization of artificial caries-like lesions: An in vitro study. Australian dental journal. 53. 34-40. 10.1111/j.1834-7819.2007.00006.x.
11. Kawai K, Heaven TJ, Retief DH. In vitro dentine fluoride uptake from three fluoride-containing composites and their acid resistance. J Dent. 1997;25(3–4):291–6.
12. Maria B, Anuradha P,Letícia C, Natalia C, Clovis P. The effect of CPP-ACP and Nd:YAG laseron the bond strength of softened dentin. Braz Oral Res. 2015; 29(1):1-7
13. Hicks J, Wu J, Catherine M F.Effect of Xylitol and Fluoride Dentifrices on Human Enamel Surfaces Following Acid-Etching: Scanning Electron Microscopic Study. Pediatr Dent Care 2016; 1(4):1-7.
14. Hicks J, Garcia-Godoy, Flaitz C. Biological factors in dental caries: role of remineralization and fluoride in the dynamic process of demineralization and remineralization (part 3). J Clin Pediatr Dent 2004; 28(3):203-214-
15. Fonseca RB, Haiter-Neto F, Carlo HL, Soares CJ, Sinhoreti MA, Puppin-Rontani RM, Correr-Sobrinho L. Radiodensity and hardness of enamel and dentin of human and bovine teeth, varying bovine teeth age. Arch Oral Biol 2008; 53: 1023-1029
16. Ionta, Franciny & Mendonça, Fernanda & Oliveira, Gabriela & Alencar, Catarina & Marques Honório, Heitor & Magalhaes, Ana Carolina & Rios, Daniela. (2013). In vitro assessment of artificial saliva formulations on initial enamel erosion remineralization. Journal of dentistry. 42. 10.1016/j.jdent.2013.11.009.
17. Khan, M. S. I., Oh, S. W.,Kim, Y. J. Power of scanning electron microscopy and energy dispersive X-ray analysis in rapid microbial detection and identifcation at the single cell level. Sci. Rep. 10, 2368. https://doi.org/10.1038/s41598-020-59448-8 (2020).
18. Scimeca, M., Bischetti, S., Lamsira, H. K., Bonfglio, R. & Bonanno, E. Energy Dispersive X-ray (EDX)

microanalysis: A powerful tool in biomedical research and diagnosis. EJH 62, 2841. https://doi.org/10.4081/ejh.2018.2841 (2018).

19. Schlueter, N., Hara, A., Shellis, R. P. & Ganss, C. Methods for the measurement and characterization of erosion in enamel and dentine. Caries Res. 45(Suppl 1), 13–23. https://doi.org/10.1159/000326819 (2011).

20. Cochrane, N. J. et al. Comparative study of the measurement of enamel demineralization and remineralization using transverse microradiography and electron probe microanalysis. Microsc. Microanal. 20, 937–945. https://doi.org/10.1017/s14319276140006 22 (2014)

21. Jayarajan J, Janardhanam P, Jayakumar P, Deepika. Efficacy of CPP-ACP and CPP-ACPF on enamel remineralization – anin vitro study using scanning electron microscope and DIAGNOdent. Indian J Dent Res 2011;22(1):77–82.

22. Halim, S. & Raafat, Rasha. (2017). Chemical and Morphological Analysis of Human Demineralized Enamel Surface By Acid And Laser Etching Following Remineralization by Mi Paste Plus. Egyptian dental journal. 63. 1425-1433. 10.21608/edj.2017.76197.

23. el Habashy, Laila & Heikal, Mona. (2020). Effectiveness of Casein Phospopeptide Amormphous Calcium Phosphate With or Without Fluoride on Reminelization of Enamel Caries- Like Lesions In Primary Teeth. Egyptian Dental Journal. 66. 799-808. 10.21608/edj.2020.24528.1041.

24. Reynolds EC CF, Shen P, Walker GD. Retention in plaque and remineralization of enamel lesions by various forms of calcium in a mouthrinse or sugar-free chewing gum. J Dent Res. 2003 82:206-11.

25. Cross KJ, Huq NL, Palamara JE, Perich JW, Reynolds EC. Physicochemical characterization of casein phosphopeptide-amorphous calcium phosphate nanocomplexes. J Biol Chem. 2005; 280:15362-9.

26. Llena C, Forner L, Baca P. Anticariogenicity of casein phosphopeptide amorphous calcium phos phate: a review of literature. J Contemp Dent Pract. 2009; 10: 1-9.

27. Reynolds EC. Anticariogenic complexes of amorphous calcium phosphate stabilized by casein phosphopeptides: a review. Spec Care Dentist. 1998 JanFeb;18:8-16.

28. Kumar VL, Itthagarun A, King NM. The effect of casein phosphopeptideamorphous calcium phosphate on remineralization of artificial caries-like lesions: an in vitro study. Aust Dent J. 2008 Mar;53:34-40.

29. Salman N, ElTekeya M, Bakry N, Omar S and El Tantawi M. Comparison of remineralization by fluoride varnishes with and without casein phosphopeptide amorphous calcium phosphate in primary teeth, Acta Odontologica Scandinavica, DOI: 10.1080/00016357.2018.1490

30. Reynolds EC. Dairy products and dental health. Proceedings of the nutrition society of Australia. 1995;19:95-102

31. Elsayad I, Sakr A, Badr Y. Combining casein phosphopeptide-amorphous calcium phospha gistic remineralization potential of artificially demineralized enamel or not? J Biomed Opt. 2009

32. Hegde AM, 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. J Conserv Dent. 2012;15:61-7

33. Holler BE, Friedl KH, Jung H, Hiller KA, Schmalz G. Fluoride uptake and distribution in enamel and dentin after application of different fluoride solutions. Clin Oral Investig. 2002 Sep;6:137-44.

34. Moezizadeh M, Moayedi S. Anticariogenic Effect of Amorphous Calcium Phosphate Stabilized by Casein Phosph peptid: A Review Article. Res.J.Biol.Sci. 2009; 4:132-6.

35. Heshmat H, Ganjkar MH, Jaberi S, Fard MJ. The effect of remin pro and “MI Paste Plus” on bleached enamel surface roughness. J Dent “Tehran”. 2014; 11:131–6.

36. Moniek W Beerens, Jacob M ten Cate, Mark J Buijs, Monique H van der Veen: Long-term remineralizing effect of “MI Paste Plus” on regression of early caries after orthodontic fixed appliance treatment: a 12-month follow-up randomized controlled trial. Eur J Orthod. 2018 Sep 28;40:457-64. doi: 10.1093/ejo/cjx08