EVALUATION OF REMINERALIZING POTENTIAL OF THREE DIFFERENT REMINERALIZING AGENTS ON ARTIFICIAL CARIOUS LESIONS: A COMPARATIVE STUDY

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Abstract

Background: Invasive therapy of carious lesions initiates a cascade of re-treatment cycles with increasing loss of dental hard tissue. Non-invasive treatment using a remineralization system aims at delaying this cascade and has gained increasing attention.

Aim: To evaluate the remineralization capability of CPP-ACP + Fluoride, Tricalcium Phosphate and xylitol herbal-based dentifrices on artificial carious lesion using Cariescan Pro device.

Material and Method: Sixty extracted molars/premolars that satisfied the inclusion criteria were divided into four groups randomly: Group I-CPP-ACP + Fluoride, II- Tricalcium Phosphate, III- Xylitol herbal-based dentifrices and IV- Control Group. The samples were prepared and immersed in a bath of demineralizing solution for 96 hours to induce artificial carious lesions. Samples were than subjected to remineralizing agents daily for four minutes, washed with distilled water followed by storage in artificial saliva for 21 days. The evaluation of samples for remineralization was done using Cariescan PRO on the 7th, 14th and 21st day.Wilcoxon sign rank test was used for intragroup comparison whereas Kruskal-Wallis and Mann-Whitney test were used for intergroup comparison.

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Results: Group I (19.33±4.67) and group II (18.73±5.14) had significantly higher amount of remineralization potential when compared to group III (27.20±7.08) and control group at 21 days and was statistically significant (*P*< 0.001). Maximum remineralization was observed with Tricalcium Phosphate at 21 days (18.73±5.14).

Conclusion: Considerable amount of remineralization of early carious lesions can be promoted by TCP and CPP-ACP-F. These are excellent delivery vehicles available in a slow release amorphous form to localize calcium, phosphate and fluoride at the tooth surface.

Key words: Enamel Lesion,Tooth Remineralization, CPP-ACPF, Tricalcium Phosphate, Xylitol, Artificial Saliva, Cariscan Pro.

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Introduction: Dental caries is a chronic disease consisting of alternating demineralization-remineralization episodes rather than a one-way decalcification process. Remineralizing agentsstop or reverse the chemical destruction of tooth enamel, thus obviating the use of restorative materials.[1]

The risk of fluoride toxicity following fluoride ingestion, when used as remineralizing agent in young children lead to innovation of materials like casein phosphopeptide amorphous calcium phosphate (CPP-ACP), fluoride-containing hydroxyapatite, bioactive glass, tricalcium phosphate (TCP), etc.that provide remineralization with less harmful effects.

CPP-ACP has been widely studied and accepted.[2] When fluoride is added to CPP-ACP it has shown higher remineralization potential.[3]TCP is a “smart” calcium phosphate that controls the delivery of calcium and phosphate ions to the teeth and works synergistically with fluoride to enhance the plaque pH, thereby initiating remineralization.[4]

The traditional medicinal method of using phytochemicals isolated from plants like Aloe barbadensis miller has strong bactericidal properties against streptococcus mutans andin combination with xylitol has shown promising result.[5]

Since there are scarce studies on comparison of these three remineralization agents, this study evaluated and compared their remineralizing capacity on artificial carious lesion.

Materials and method: The proposed study was conducted as an in vitro study after prior approval and consent from the Ethical and Research Committee of the Karnavati University. In this study, 60 permanent extracted teeth were used which included caries-free maxillary/mandibular premolars and molars removed for orthodontic and impaction reasons respectively.

The exclusion criteria involved:

- Teeth with dental caries,
- Teeth with moderate to severe periodontal disease,
- Teeth with hypoplastic lesions,
- Teeth with fracture of either crown or root,
- Teeth with developmental defects or any other crown deformities,
- Teeth with restorations.

The storage of teeth was done in 10% formalinafter extraction. The selected teeth were properly washed and cleaned to remove all the soft tissue, debris and calculus. A micromotor, contra-angled handpiece, polishing cup, and polishing paste were used to polishbuccal surfaces of all the teeth. The cleaned extracted teeth were randomized into four groups using computer randomization method.

Four study groups were:

Group I: CPP-ACP + Fluoride (GC Tooth Mousse plus)

Group II: Tricalcium Phosphate (Clinpro Tooth crème)
Group III: Xylitol herbal based (Mamaearth™) dentifrices
Group IV: Control Group (Artificial Saliva)

The teeth were numbered from one to fifteen in all the groups,on root portion of the extracted tooth. The extracted teeth were than painted using a brush with acid-resistant nail varnish, leaving an enamel window of 4 mm × 4 mm on the center of the buccal surface of each tooth. After that, nail varnish was allowed to dry at room temperature and a second coat was applied.Four different color nail varnishes were used for ease of identification. It was allowed to dry for 30 minutes and after that the baseline reading of enamel specimens was assessed using CariescanPro™ device analyzing the central point of the exposed surface.The teeth were then immersed in container containing the demineralizing solution followed by incubation at 37°C for 96 hours which resulted in artificial caries-like lesion.

Composition of the demineralizing solution used was:

- 2.2 mM $CaCl_2 \cdot 2H_2O$.
- 2.2 mM $NaH_2PO_4 \cdot 7H_2O$.
- 0.05 M lactic acid and 0.5 ppm fluoride ion.
- The final pH was adjusted to 4.52 at 37°C with 50% NaOH.

After 96 hours of incubation in the demineralizing solution, the teeth were rinsed with distilled water, dried with the help of an air syringe and stored in four separate clean containers with normal saline until they were further evaluated. Readings of the samples were taken using the device Caries can PRO. Samples showing an instantaneous value of 21 and higher on the digital display were used for further evaluation as these values are indicative of a subsurface lesion on the tooth.

Following that, samples in each group were treated with respective remineralizing agent using a cotton applicator tip every 24 hours for 7 days (excluding the control group). Samples that were present in experimental groups were gently rubbed with the remineralizing agent for 4 minutes, rinsed with distilled water, and placed in artificial saliva. In the control group, samples were rinsed only with distilled water and placed in artificial saliva. Every 24 hours, artificial saliva was replenished just before freshly treated samples were immersed.

After seven cycles of remineralization, the surface of each sample was assessed using Cariescan Pro to note the values.

Remineralizing agents were applied again following the same protocol and the surfaces were re-assessed with Cariescan Pro on 14th and 21st day.

Statistical analysis: Statistical analysis was performed with SPSS software (version 21.0). Wilcoxon sign rank test was used to assess the difference at various intervals for intragroup comparison. The intergroup comparison at various intervals was done using Kruskal-Wallis test and intergroup comparison of variables at different time intervals was done using Mann–Whitney test.

Results: When an intragroup comparison of time intervals in each group was done, significant difference in Cariescan values were observed in all groups except in Control group (Table 1).

On intergroup comparison, it was observed that at 21 days of remineralization, there was a significant difference in Cariescan values in all the groups with a *P* value < 0.001.

At 7th, 14th and 21st day, Group II showed maximum remineralization (Table 2). When group I and group II were compared, the mean values of remineralization at 21st day was more for Group II (18.73) than Group I (19.33), although there was no statistically significant difference between them. Between Group I and III, the mean values of remineralization was more for Group I.

When the three study groups (group I, II and III) were compared with the control group

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(group IV), there was a significant difference in the Cariescan Pro values on the 14th and 21st day of the remineralization (Table 3).

Table 1: Intragroup comparison assessing the difference between various time intervals using Wilcoxon sign rank test

	Group I		Group II		Group III		Group IV	
	Z	*P* value	Z	*P* value	Z	*P* value	Z	*P* value
Demineralization-Baseline	-3.41	0.001*	-3.41	0.001*	-3.41	0.001*	-3.41	0.001*
7 Days-Baseline	-3.41	0.001*	-3.41	0.001*	-3.41	0.001*	-3.41	0.001*
14 Days-Baseline	-3.41	0.001*	-3.41	0.001*	-3.42	0.001*	-3.41	0.001*
21 Days-Baseline	-3.30	0.001*	-3.41	0.001*	-3.41	0.001*	-3.41	0.001*
7 Days-Demineralization	-3.42	0.001*	-3.25	0.001*	-3.43	0.001*	-2.97	0.003*
14 Days-Demineralization	-3.41	0.001*	-3.41	0.001*	-3.42	0.001*	-3.22	0.001*
21 Days-Demineralization	-3.41	0.001*	-3.41	0.001*	-3.41	0.001*	-3.33	0.001*
14 Days-7 Days	-3.43	0.001*	-3.41	0.001*	-3.43	0.001*	-2.43	0.015*
21 Days-7 Days	-3.42	0.001*	-3.41	0.001*	-3.43	0.001*	-3.25	0.001*
21 Days-14 Days	-3.42	0.001*	-3.41	0.001*	-3.32	0.001*	-2.81	0.005*

**P*< 0.05 statistically significant, *P*> 0.05 non-significant, NS Z-score <-1.96 and >1.96 are considered significant at *P*-value threshold of 0.05

Table 2: Intergroup comparison at various intervals using Kruskal-Wallis test.

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Time interval	Groups	N	Mean (SD)	Range	Median (IQR)	Chi-square value	*P* value
Baseline	I	15	8.13(5.83)	2-19	6(3.5-12.5)	0.208	0.976(NS)
	II	15	8.67(6.31)	2-19	6(3.5-14.5)		
	III	15	8.33(5.16)	1-19	8(5.5-11.5)		
	IV	15	8.20(3.67)	2-15	8(5.5-10.5)		
After Demineralisation	I	15	36.73(7.34)	24-47	35(32.5-45)	0.246	0.970(NS)
	II	15	36.33(8.74)	25-48	32(29.5-45)		
	III	15	36.07(8.38)	26-49	33(29-42.5)		
	IV	15	36.60(4.86)	27-43	37(36-39.5)		
7 Days	I	15	32.47(7.98)	20-44	30(27-40.5)	2.167	0.539(NS)
	II	15	32.20(7.99)	21-44	33(25.5-40)		
	III	15	33.13(8.41)	24-47	31(26.5-39.3)		
	IV	15	35.67(4.43)	27-42	36(35-39)		
14 Days	I	15	26.47(6.67)	18-39	24(22-30.5)	15.236	0.002*
	II	15	26.07(6.79)	17-36	25(20.5-33)		
	III	15	30.07(7.71)	22-44	27(23.5-33.5)		
	IV	15	35.00(4.87)	25-42	36(34-38)		
21 Days	I	15	19.33(4.67)	14-29	17(16-22)	32.704	<0.001*
	II	15	18.73(5.14)	13-27	17(14-23.5)		
	III	15	27.20(7.08)	20-39	25(21.5-31.5)		
	IV	15	34.27(4.65)	25-41	35(33-37.5)		

*P< 0.05 statistically significant, P > 0.05 non-significant, NS

		Baseline	Demineralization	7 Days	14 Days	21 Days
Group I vs Group II	U Statistics	110.50	103.50	104.00	103.50	95.00

	p value	0.934(NS)	0.708(NS)	0.724(NS)	0.708(NS)	0.466(NS)
Group I vs Group III	U Statistics	105.50	98.50	108.50	78.00	36.00
	p value	0.770(NS)	0.560(NS)	0.868(NS)	0.151(NS)	0.001*
Group I vs Group IV	U Statistics	110.00	104.00	83.50	38.00	5.00
	p value	0.632(NS)	0.724(NS)	0.228(NS)	0.002*	<0.001*
Group II vs Group III	U Statistics	109.50	109.50	103.50	81.00	44.50
	p value	0.901(NS)	0.901(NS)	0.708(NS)	0.191(NS)	0.005*
Group II vs Group IV	U Statistics	107.50	111.50	87.00	29.00	4.00
	p value	0.835(NS)	0.967(NS)	0.289(NS)	0.001*	<0.001*
Group III vs Group IV	U Statistics	107.50	103.50	82.50	61.00	51.00
	p value	0.835(NS)	0.708(NS)	0.211(NS)	0.032*	0.010*

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Table 3: Intergroup comparison of variables at different time intervals using Mann-Whitney Test

*P< 0.05 statistically significant, P> 0.05 non-significant, NS

Discussion: The key to dental caries prevention is the balance between remineralization and demineralization. Prevention has emerged as a new era in recent times, which focuses on the "minimally invasive" approach that emphasizes on early detection of these carious lesions.Remineralization has remained the major area of investigation, but still, it is difficult to accurately define the efficacy and success of various remineralization methods.[6]

CarieScan PRO is an example of one modern diagnostic tool that works on the principle of difference in electrical conduction between healthy and carious tissue for caries detection. This system uses multiple low voltage frequencies and Alternating Current impedance spectroscopy.[7]

In this study three remineralizing agentsused were namely CPP-ACP + Fluoride, Tricalcium Phosphate and Xylitol herbal-baseddentifrices. The mechanism of action of these agents were different but the results obtained indicate that all the three test groups enhanced remineralization. In our study, the enamel samples were demineralized for 96 hours. Artificial enamel subsurface lesion was produced using buffered acidic solution which causes dissolution of HA crystals resulting in demineralization of enamel. Once the demineralization of samples was done they were subjected to remineralization for a period of 21 days. The samples were evaluated for remineralization and demineralization using Cariescan PRO. The period for pH cycling was 21 days, to provide sufficient time for the agents to act on the demineralized enamel specimen. The basic process of remineralization requires diffusion of calcium and phosphate ions from

saliva and other topical sources to create an, acid-resistant, hyper mineralized, fluorapatite like layer on the crystal remnants that serve as remineralization nuclei.

The detection of demineralization can be done by two methods: (1) non-invasive and (2) invasive. Enamel white spot lesions are not detectable visually until they have progressed 200–300 µm into the enamel. Quantitative light fluorescence (QLF), electrical impedance spectroscopy, fiberoptic transillumination, optical coherence tomography, laser fluorescence (DIAGNOdent®), and SEMare some of the non-invasive diagnostic methods available.[2]

In this study CarieScan Pro™ was used to perform the quantitative analysis. This device uses electrical impedance spectroscopy (EIS) to quantify the levels of demineralization of the enameland is able to detect and monitor early mineral losses. The principle of bioimpedance for the detection of incipient carious lesion is based on the fact that the enamel has less porosity and a little amount of fluid and so it is a poor conductor.[8]

From the comparison of the mean remineralization value of CPP-ACPF (19.33), TCP + Fluoride (18.72), Herbal dentifrice (27.20) to its demineralization value (36.73), (36.33), (36.07) respectively, it is clear that a notable amount of remineralization had taken place. On comparingthe mean remineralization value of artificial saliva (36.60) with its mean demineralization value (34.27) it was noted that there was a certain amount of remineralization; which indicates that saliva by itself has some remineralization capability. On comparing CPP-ACPF and TCP + Fluoride to Herbal dentifrice, a significantly higher amount of remineralization was seen with CPP-ACPF and TCP+ Fluoride. Although saliva has some remineralization capacity, it is unable to significantly enhance the levels of calcium and phosphate release on its own. For mineral deposition to take place within the body of the lesion, calcium and phosphate ions must first penetrate the surface layer of the enamel. It was also noted that TCP + fluoride was found to be more effective than CPP-ACP + fluoride (but this difference was not significant). The reason for better remineralization capacity of TCP can be higher concentration of calcium ions which has been suggested by Karlinsey and Mackey in their studystating that the protective barriers in TCP are broken by salivary moisture from the tooth, making more fluoride ions, calcium, and phosphateavailable to the surface of the teeth.[9]

The results of this study were comparable to those of the study by Patil et al., who compared CPP-ACP, CPP-ACPF, and TCP and found that TCP had the highest remineralization efficacy.[10]According to Damyanova et al., Clinpro white varnish with TCP is effective in reducing subsurface layer demineralization and promoting remineralization of the surface and subsurface enamel layers.[11]In another study, AlAmoudi et al.[12]found that adding fTCP to a fluoride varnish significantly increases the varnish's ability to protect deciduous teeth.The results of both the investigations mentioned above are consistent with those of the present study.

In a study done by Bhat et al.[13], the finding revealed that both CPP-ACPF (Tooth Mousse Plus™) and tricalcium phosphate (customized dentifrice) showed almost similar remineralization potential but CPP-ACPF showed significantly more amount remineralization which is contradictory to our study.In another study by Thimmaiah C et al., EDX analysis showed that with CPP-ACPF the percentage gain of calcium and phosphate was maximum after remineralization when compared to TCP and nanohydoxyapetite.[1] The result of both these studiesare in contrast to the results of our study. The difference in results could be use of different demineralizing solution, duration of remineralizing agent

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applied or the difference in caries diagnostic aid.

The device used in our study was Cariescan PRO which is less precise as compared to SEM and hence could have affected the result obtained. Also in vitro remineralization can be quite different from the dynamic and complex biological systems, that normally occurs in oral cavity in vivo which might have affected the results.

Conclusion: It can be concluded from the present study that all the three remineralizing agents were effective as remineralizing agent as compared to artificial saliva. CPP-ACP along withfluoride and Tricalcium Phosphatecontaining toothpastes have a promising role when applied topically in managing incipient carious lesion.

References

1. Thimmaiah C, Shetty P, Shetty SB, Natarajan S, Thomas NA. Comparative analysis of the remineralization potential of CPP-ACP with Fluoride, Tri-Calcium Phosphate and Nano Hydroxyapatite using SEM/EDX - An *in vitro* study. J Clin Exp Dent. 2019 Dec 1;11(12): e1120-e1126.
2. Jayarajan J, Janardhanam P, Jayakumar P, Deepika. Efficacy of CPP-ACP and CPP-ACPF on enamel remineralization - An in vitro study using scanning electron microscope and DIAGNOdent®. Indian J Dent Res. 2011; 22:77-82.
3. 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; 9:58-63.
4. Reynolds EC. Calcium phosphate-based remineralization systems: scientific evidence? Aust Dent J. 2008; 53:268-73.
5. Ranjana BS, Chowdhary N, Kiran N K, Chaitan S M, Reddy V R, Prabahar T. Remineralizaion efficacy of nonfluoride versus herbal based pediatric dentrifice in demineralized primary teeth. J Indian Soc PedodPrev Dent 2021:39:67-73.
6. Abou Neel EA, Aljabo A, Strange A, Ibrahim S, Coathup M, Young AM, et al. Demineralization-remineralization dynamics in teeth and bone. Int J Nanomedicine. 2016;11:4743-63.
7. Kockanat A, Unal M. In vivo and in vitro comparison of ICDAS II, DIAGNOdent pen, CarieScan PRO and SoproLife camera for occlusal caries detection in primary molar teeth. Eur J Paediatr Dent. 2017 Jun;18(2):99-104.
8. Kühnisch J, Heinrich-Weltzien R, Tabatabaie M, Stösser L, Huysmans MC. An in vitro comparison between two methods of electrical resistance measurement for occlusal caries detection. Caries Res. 2006;40(2):104-11.
9. Karlinsey RL, Mackey AC. Solid-state preparation and dental application of an organically modified calcium phosphate. J Mater Sci 2009;44(1):346–349.
10. Patil N, Choudhari S, Kulkarni S, Joshi SR. Comparative evaluation of remineralizing potential of three agents on artificially demineralized human enamel: An in vitro study. J Conserv Dent 2013; 16:116-20.
11. Damyanova D. In Vitro Study of the Effects of Fluoride-Releasing Dental Materials on Re-Mineralization of Deciduous Teeth. IOSR J Dent Med Sci 2016 July;15(7):98–101.
12. AlAmoudi SA, Pani SC, et al. The effect of the addition of tricalcium phosphate to 5% sodium fluoride varnishes on the microhardness of enamel of primary teeth. Int J Dent 2013 May 28; 2013:4.
13. Arjun DS, Bhat SS, Hegde SK, et al. Comparative Evaluation of Two Remineralizing Agents on Artificial Carious Lesion Using DIAGNOdent. Int J Clin Pediatr Dent 2021;14(2):192–195.

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