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Original Research Article

Laser – fluorescence based evaluation of remineralization in artificial enamel lesions using three commercially available re-mineralizing agents – An invitro study

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ABSTRACT

Aim & Objective: The objective of this in-vitro study is evaluation of remineralization potential of three remineralization agents- Tricalcium phosphate fluoride(TCP-F), Calcium sucrose phosphate(CaSP), Casein phosphopeptide- amorphous calcium phosphate(CPP-ACP) in artificial enamel lesion using laser fluorescence based DIAGNOdent(KaVo).

Materials and Methods: 30 single rooted premolars were selected. Samples were randomly divided into 3 groups with 10 samples each. Baseline values were recorded before any intervention. A 4mmx4mm window was prepared on the buccal surface which was then subjected to demineralization for 96 hr at 37°C. DIAGNOdent values were recorded after demineralization. Remineralization was done using Group I TCP-F, Group II CaSP, Group III CPP-ACP remineralizing agents. The samples were subjected to DIAGNOdent analysis after 72hrs, 7days, 14 days.

Statistical analysis: The data was analyzed with analysis of variance (ANOVA) and Post Hoc test.

Results: All three groups showed significant difference in DIAGNOdent values at baseline, post demineralization and post remineralization. TCP-F group showed maximum remineralization followed by CaSP and CPP-ACP groups.

Conclusion: DIAGNOdent is a reliable non-invasive caries detecting device to measure early demineralization. TCP-F, CaSP, CPP-ACP are proven to possess remineralization properties.

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1. Introduction

Dental caries is a slow progressive irreversible microbial disease of the teeth, which leads to cavitation following the demineralization of the inorganic portion and destruction of the organic substances of the tooth. The reduction in pH of the saliva below the “critical pH,” due to the organic acids produced by the caries causing bacteria lead to abnormal loss of minerals from the enamel surface or subsurface

known as demineralization.¹ Remineralization takes place when calcium, phosphate and fluoride ions are directly available in the oral environment and early dental caries can be arrested or repaired by enhancing the natural capacity of saliva to re-mineralize teeth.² With the advancement in early caries management, newer fluoride and non-fluoride containing remineralizing agents have been introduced in the market as such as TCP-F, CPP-ACP, bioactive glass and CSP. Therefore, by using DIAGNOdent pen the current study compares and evaluates the effectiveness of the three

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newer remineralizing agents TCP-F, CaSP, and CPP- ACP in remineralizing initial enamel caries.

2. Materials and Methods

Thirty maxillary and mandibular non-carious single-rooted premolars that were extracted for periodontic and orthodontic reasons were selected for the study. Teeth with no evidence of caries, cracks or white spot lesion on visual inspection, and showed DIAGNOdent score: 0-7 were included, whereas teeth that had restorations or developmental defects and DIAGNOdent score more than 7 were excluded. All teeth were sterilized and stored in 1% thymol solution for further preparation. The buccal surfaces of all teeth were polished, and all teeth were sectioned 1 mm below cemento-enamel junction. They were then embedded in acrylic blocks such that only crown portions were exposed. An area of 4 mm × 4 mm (window) was marked on the buccal surfaces of teeth samples, coated with nail varnish except for the window, which was analyzed for the baseline values using DIAGNOdent prior to demineralization and remineralization. The samples were randomly divided into three groups based on the application of the remineralizing agents used as follows:

1. Group 1 (n = 10) – Remineralized with TCP-F (Clinpro tooth creme®, 3M ESPE)
2. Group 2 (n = 10) – Remineralized with CaSP (Toothmin®, Abbott Healthcare)
3. Group 3 (n = 10) – Remineralized with CPP-ACP (Recaldent, GC Tooth Mousse®.)

Demineralization procedure

The samples of each individual group were stored in 200 ml of freshly prepared demineralization solution comprising 2.2 mM calcium chloride, 2.2 mM potassium hypophosphate, and 0.05M acetic acid (pH 4.4) with 1 M potassium phosphate for a period of 96 h. The bottles were placed in an incubator at 37°C. The samples were removed, rinsed, and air-dried to detect white spot lesion.³

2.1. Remineralization procedure

According to the method described by Sato et al.,⁴ the artificial saliva was prepared. The pH was set at a level of 7.2. Three artificial mouth models were made, one for each group. Each sample was mounted in the base of the individual compartment of a polyvinyl box, such that their buccal surface faced upward; a circular opening was made in the walls of the compartment. Exit hole was made for the exit pipe, which finally drained into a collector. For each compartment, a controlled dropper was fixed and adjusted on the box lid such that it delivers 5 drops of solution/min on the window area. The controlled droppers of each compartment were interconnected using polyvinyl tubes with the help of two-way plastic connectors. All

artificial mouth models were prepared with individual inlet and outlet. The whole setup was placed in the incubator, temperature of which was maintained at 37°C, and there was a continuous flow of saliva with intermittent flow of buffer solution simulating oral conditions. Continuous exposure of the samples to artificial saliva with intermittent exposure to buffer solution 3 times daily for a period of 15 min each for 14 days was maintained, resembling the pH changes occurring in the mouth.

The teeth samples in each respective group were remineralized by the application of TCP-F, CaSP and CPP-ACP using powered toothbrush (oral B) twice daily for 3 min onto the tooth surface window every 24hrs for 14 days. Post remineralization DIAGNOdent readings of samples were recorded 72hrs, 7days and 14 day interval.

3. Results

Statistical analysis was done by one-way ANOVA and post hoc Bonferroni test where $p < 0.05$ was statistically significant. All the groups showed statistically significant remineralization. DIAGNOdent values show statistically significant difference from baseline, post demineralization and after remineralization $p < 0.001$. Remineralization post 7th and 14th of treatment showed statically different results in post hoc Bonferroni test. TCP-F is found to have better remineralization capacity than other groups.

4. Discussion

Non invasive methods for detection of early stage of caries and use of commercially available remineralization agents have the potential to be a major advance in the clinical management of the disease and decrease the burden of oral disease worldwide.

This study attempted to recreate the clinical intraoral situation with changes in Ph values to investigate the remineralization potential of the commercially available agents. Twice daily application of the toothpaste was done as it concluded in study done by Lata et al.⁵ that effect of remineralization on demineralised enamel is more with extended period of time kaveri et al., reviewed the ideal brushing time and concluded that it should last for 2-3 min. Hence the remineralizing agents were applied to the teeth surface over period of 3 mints.

KaVo DIAGNOdent (Germany) is a non-invasive laser-based instrument that emits a laser light with a wavelength of 655 nm used to detect early caries as it measures early demineralization of tooth. The organic and inorganic materials in the teeth absorb the laser light and reemit it as fluorescence within the infrared region. In presence of caries the light with a higher wavelength is reemitted and the changes are registered in a digital number scale, with an audible sound indicating the fluorescence increase. After demineralization the DIAGNOdent values increased and

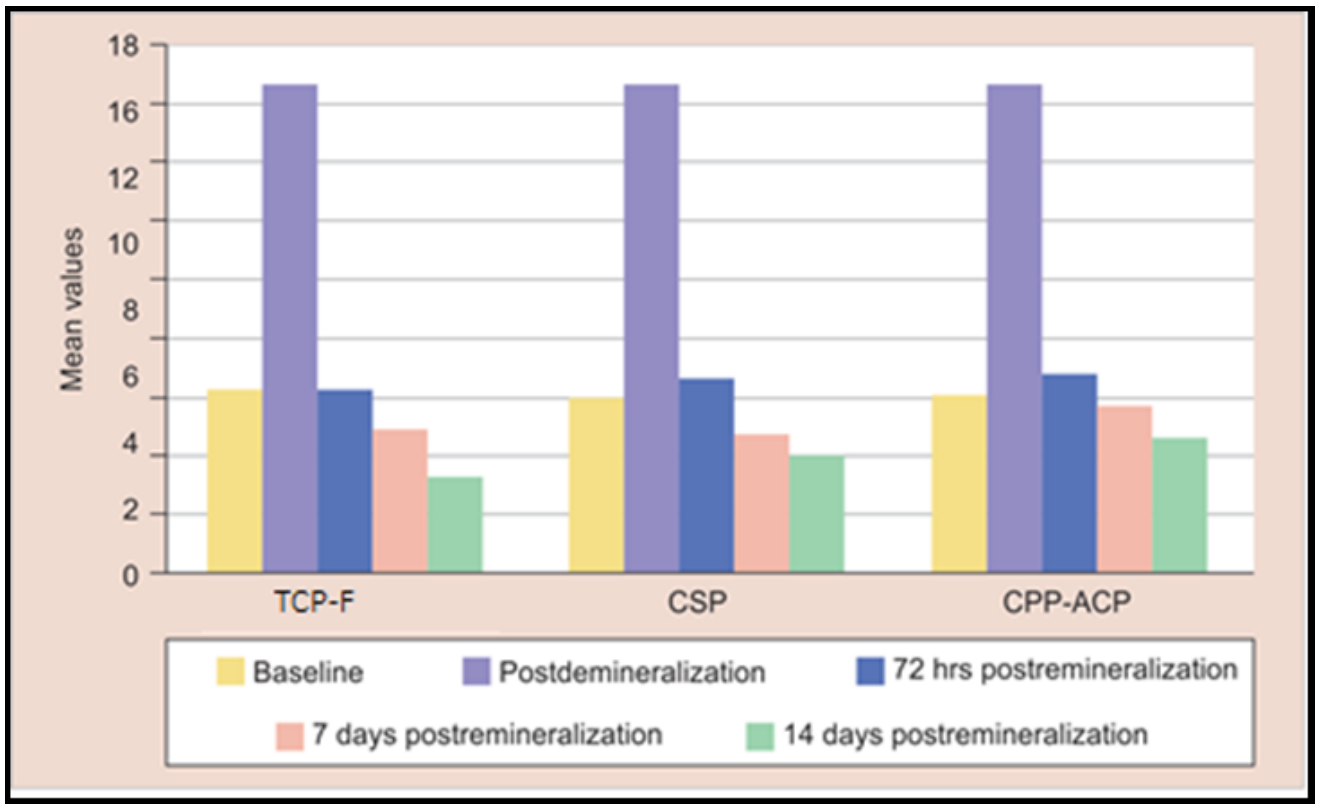


Fig. 1: The mean values of DIAGNOdent readings.

Table 1: ANOVA test

	df	F	Sig.
Baseline	Between groups	2	0.248
	Within group	27	
Demineralization	Between groups	2	0.019
	Within group	27	
Remineralization 72 hours	Between groups	2	0.984
	Within group	27	
Remineralization 14 hours	Between groups	2	5.771
	Within group	27	0.008
	Between groups	2	7.833
	Within group	27	0.002

Table 2: Post hoc tests: Multiple comparison-Bonferroni

Dependent variable	(I) group	(J) group	Sig.
Remin_7 days	TCP-F	CSP	1.000
		CPP-ACP	0.048
		SDF	1.000
	CSP	CPP-ACP	0.010
		SDF	0.048
		CSP	0.010
Remin_14 days	TCP-F	CSP	0.126
		CPP-ACP	0.001
		SDF	0.126
	CSP	CPP-ACP	0.235
		SDF	0.001
		CSP	0.235

post remineralization the values decreased. Comparative results were seen in studies conducted by Al-Khatteb et al.⁶ pai et al.⁷ & Patil et al.⁷ who used DIAGNOdent to assess remineralization.

Fluoride has always been used in dentistry as it strengthens the tooth by its ability to promote net remineralization. Salivary calcium concentration is inversely related to solubility of enamel and the ability of Fluoride ions to drive a net remineralization process is limited by the availability of calcium and phosphate ions. So an adequate supply of salivary calcium and phosphate is a limiting factor to avail the benefits of topical fluoride application.⁷

Clinpro tooth crème contains 0.21% sodium fluoride and functionalized tricalcium phosphate (TCP) which provides a continuous reservoir of ions and controlled supply of calcium and phosphate ions. Functionalized tricalcium phosphate, has another benefit as it enhances the action of fluoride on enamel surfaces. TCP can provide optimal benefits when delivered in a neutral pH whereas other calcium phosphate additives may require an acidic pH to work.⁷ Therefore, TCP ingredient can enhance mineralization and help build high quality, acid resistant mineral without the dependence for high levels of calcium.⁷ Clinpro contains 5000 ppm sodium fluoride with TCP and is superior to CPP-ACP and varying concentrations of topical fluoride.⁷ The present study showed that TCP-F is a potent remineralizing agent. The post remineralizing values approached the base line values after 72hrs of remineralization. Maximum remineralization was found during 7th & 14th day which is statistically significant (Tables 1 and 2).

Calcium sucrose phosphate based on Anticay® works by creation of an aqueous solution containing high concentration of calcium (10–12%) and phosphate (8–10%) without precipitation of the ions. It also inhibits the formation of plaque and is a potent water soluble remineralizing agent providing adequate supply of free calcium and phosphate ions several times higher than normally present in saliva.⁷ In an in vitro study comparing the effect of GC Tooth Mousse (CPP-ACP) and Toothmin toothcream (CaSP) on microhardness of bleached enamel surface Kaur et al.⁷ found that remineralization was numerically better in Toothmin group, which showed increased microhardness.⁷ The results of this study corroborate with an in vitro study conducted by Raghu et al.⁷ & found that Toothmin showed remineralizing potential and increased microhardness of enamel. Calcium sucrose phosphate group showed a significant difference in postdeminerization and postremineralization values in this study with maximum remineralization occurring during 7th and 14th day (Figure 1 and Tables 1 and 2).

The concept of using CPP-ACP containing milk protein CPP along with ACP decreases tooth enamel demineralization and promotes enamel remineralization⁷

Casein phospho peptide–amorphous calcium phosphate is highly soluble in water providing high concentrations of free calcium and phosphate resulting in net remineralization of enamel. CPP-ACP has shown to reduce the streptococcus mutans biofilm development on GIC restoration and also disrupt the established biofilms inhibits new plaque accumulation on enamel. CPP-ACP favors remineralization although the properties are inferior to TCP-F (Figure 1 and Tables 1 and 2)

Though the results of present study demonstrated that the given remineralization agents can induce remineralization of the early enamel lesions inspite of differences in composition and mechanism of action. Limitation of the study were that although surface remineralization was confirmed, enamel subsurface remineralization was not evaluated and remineralization in vitro may be quite different when compared with dynamic functional biological system in the mouth. To arrive at a comprehensive result the findings of in-vitro research need to be compared with the in-vivo studies so that early enamel caries remineralization protocol can be established.

5. Conclusion

The results of the present study can help in mineralization and management of hypocalcified lesions, desensitization of exposed dentine affected by dental erosion and after debonding of brackets in lieu of completion of orthodontic treatment. TCP-F based products performed better than CPP-ACP, CaSP based products in remineralizing artificial enamel caries .

6. Conflicts of Interests

The authors have no financial interests or conflicts of interests.

7. Source of Funding

None.

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