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Comparative Analysis of Remineralizing Potential of Three Commercially Available Agents- An in Vitro Study

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Abstract:
Background: *With recent advances in the field of caries research and diagnosis it is possible to remineralize early carious lesions or white spot. The present in vitro study was carried out to evaluate and compare the remineralization of artificially created subsurface enamel lesion, using three different remineralizing agents.*
Method: *60 enamel specimens were divided into 3 groups, with 10 control and 10 study specimens. After immersion in demineralizing solution for 48 hours the enamel specimens were treated with following remineralizing agents, Group A- mousse containing Casein phosphopeptide amorphous calcium phosphate (CPP-ACP), Group B- dentifrice containing functionalized tricalcium phosphate (fTCP) with 0.21% sodium fluoride, and Group C- a dentifrice containing Novamin. After 15 days of treatment, all the specimens were indented to evaluate Vickers microhardness of enamel specimens using Reicherts microhardness testing machine. The data was analysed using paired t-test and ANOVA.*
Results:*In all the three groups, there was statistically significant difference in microhardness values of enamel specimens before and after treatment with the agents ($p < 0.05$). Mean increase in microhardness value was more in Group 2 (f-TCP with 0.21% sodium fluoride). No significant difference was observed between the mean increase in microhardness across CPP-ACP, fTCP +0.21% sodium fluoride and novamin groups ($p > 0.05$).*
Conclusion: *The microhardness of enamel specimens significantly increased after treatment with remineralizing agents for 15 days. Thus, all the three agents are effective in remineralizing the enamel subsurface lesion.*
Keywords: *CPP-ACP, f-TCP, Novamin, microhardness, remineralization.*

I. Introduction

After the eruption of teeth, enamel undergoes post-eruptive maturation, which is characterized by multiple demineralization – remineralization cycles[1]. A shift in the normal equilibrium conditions towards demineralization for longer period of time results in white spots- the earliest clinical manifestation of a carious process[2]. Conventional white spot management protocols included fluoride application. The recent trend in management of early carious lesion is remineralization of lesion by delivering calcium, phosphate and fluoride ions and shift the equilibrium in favour of remineralization. At present, there are different calcium-phosphate delivery systems available which can remineralize the enamel subsurface lesion. Several studies have been conducted to test the efficacy of remineralizing agents such as CPP-ACP, functionalized tricalcium phosphate (f-TCP), etc. However, as per the published data available, there is no available literature comparing remineralizing potential of all the three agents, which were used in the present study. The aim of this in vitro study was to compare remineralization potential of three different remineralizing agents, CPP-ACP, f-TCP and Novamin using microhardness test.

II. Material And Methods

60 enamel specimens were prepared from 30 extracted permanent premolars with intact coronal structures. The buccal and the lingual surface of enamel was prepared and polished by successive grinding of the exposed enamel surface using 200,400,800,1000,1200 grit abrasive paper. The prepared surface was polished for 2 reasons: (1) enamel surface exposed was never subjected to the natural demineralization-remineralization process in the oral cavity, (2) a flat polished surface was essential for getting accurate reading of the diagonal lengths of the indentation.

The specimens were then placed in the demineralizing solution containing 20ml of acid buffer containing 2mmol/l Ca, 2mmol/l Po4 and 0.075 mol/l acetate at pH 4.3 for 48 hours, to produce artificial carious lesions. After demineralization, the specimens were then randomly assigned to 3 groups. Each tooth was sectioned into buccal and lingual halves using low speed diamond disc. Both the buccal and lingual half of each tooth was mounted in cold cure acrylic resin, such that the prepared enamel surface is exposed (Fig. 1). Buccal and lingual halves belonging to the same tooth were assigned the same number after mounting. One half was randomly selected as control (not treated with any agent) and the other half as study which was treated with remineralizing agent. Each agent was applied on the selected surface for 3 minutes daily for 15 days.

1. GROUP A – treated with 10% CPP-ACP.
2. GROUP B – treated with 0.21% sodium fluoride – tricalcium phosphate. (Clinpro tooth crème).
3. GROUP C – treated with bioactive glass containing dentifrice.

After treatment, each specimen was rinsed with distilled water and stored in artificial saliva. After a period of 15 days, the specimens were sent for evaluation of microhardness using Vickers hardness testing machine. Each surface was indented under 50 grams for 20 seconds. The diagonal length of the indentation was measured and microhardness value was calculated using formula:

$$HV = \frac{1854.4\ p}{d^2}$$

p is the load applied and d is diagonal length.

For each specimen, the control and the study samples were indented together in a single session. Hence, the microhardness of the non-treated (control) surface acted as baseline and the difference in microhardness between the study and control samples, denoted the net gain in minerals by the surface. The data gathered was analysed and compared statistically using paired t-test and ANOVA test.

III. Results

The microhardness values of the enamel specimens in the control group (after demineralization) were in the range of 179 - 186 VHN. Thus, no significant difference was observed in the control specimens **(TABLE 1)**. After treatment with remineralizing agent of respective group, the enamel microhardness increased considerably and was in the range of 270 – 290 VHN. The data obtained was analysed using Factorial Anova and paired t test on SPSS Software. Higher mean value was recorded in Group B (fTCP + 0.21% sodium fluoride) followed by Group A (CPP-ACP) and Group C (Novamin) respectively, but the difference in mean values between the groups was not statistically significant (P>0.05) **(TABLE 2)**. In each group, considerable increase was seen in the microhardness after treatment as shown in **TABLE 3 & Fig. 2**.

Table 1: Mean values of Microhardness (VHN) in control samples across the groups

Group	N	Mean	Std. Deviation	Std. Error	P Value
Group A	10	179.60	10.72	3.39	0.846
Group B	10	181.50	13.03	4.12	
Group C	10	182.50	10.12	3.20	

Table 2: Mean values of Microhardness (VHN) in study samples across the groups

Group	N	Mean	Std. Deviation	Std. Error	P-Value
Group A	10	284.90	46.38	14.67	0.633
Group B	10	289.70	38.20	12.08	
Group C	10	273.60	28.34	8.96	

Table 3: Comparison between control & study samples within each group (t-test)

Group	Samples	Mean (VHN)	Std dev	Mean difference (VHN)	P-Value
Group A	Control samples	179.60	10.72	105.300	<0.001*
	Study samples	284.90	46.38		
Group B	Control samples	181.50	13.03	108.200	<0.001*
	Study samples	289.70	38.20		
Group C	Control samples	182.50	10.12	91.100	<0.001*
	Study samples	273.60	28.34		

IV. Discussion

In the present study, three different agents with different chemical composition were used, which deliver calcium – phosphate ions favouring remineralization. The agents containing CPP-ACP, f-TCP with 0.21% sodium fluoride and Novamin (Bioglass) respectively were used.

The new phosphopeptide isolated from the milk protein casein, complexed with calcium phosphate [Casein phosphopeptide – Amorphous calcium phosphate (CPP – ACP) nanocomplexes], has recently been shown to be efficacious in both the prevention and reversal of enamel subsurface lesions in caries models[3,4,5,6]. The CPP binds to form nanoclusters of calcium and phosphate ions in solutions to form highly soluble and bioavailable nanocomplexes. Recently, it has been shown that the CPP – ACP nanocomplexes are superior to other forms of calcium phosphate in remineralizing enamel sub-surface lesions in situ[7]. Casein phosphopeptide (CPP) has been shown to not only stabilize amorphous calcium phosphate (ACP), but also to deliver and localize ACP at the tooth surface[8,9].

Fluoride ions promote the formation of fluorapatite in enamel in the presence of calcium and phosphate ions produced during enamel demineralization. Fluoride ions can also drive the remineralization of previously demineralized enamel if enough salivary or plaque calcium and phosphate ions are available when the fluoride is applied. Thus, the f-TCP + 900 ppm of NaF containing dentifrice has an added advantage of fluoride in addition to novel functionalized tricalcium phosphate. The f-TCP contains 2 % sodium lauryl sulphate, which prevents calcium phosphate reaction with fluoride and formation of calcium fluoride. As a result, fluoride, calcium and phosphate are available in aqueous form for remineralization process[10].

NovaMin is a bioactive glass-ceramic material, which falls into a class of newer agents that provide calcium and phosphate upon reaction. In NovaMin, the active ingredient is a calcium sodium phosphosilicate (CSP) that reacts when exposed to aqueous media and provides calcium and phosphate ions that form a hydroxy-carbonate apatite (HCA) with time. The use of Novamin for management of dentin hypersensitivity is well documented. However, only a few studies are available which have assessed the remineralizing potential of Novamin on enamel.

Enamel demineralization and remineralization is a surface phenomenon and occurs at surface and subsurface level. The surface microhardness measurement therefore gives us information about surface integrity and caries progression[11]. The present study was conducted using this simple and rapid method of surface indentation for the purpose of evaluating demineralization- remineralization of enamel surface. The indentation will be deeper in demineralised enamel and subsequent diagonal length will increase **(Fig 3)**. After treatment the remineralised enamel resist the indentation force and the indentation depth and diagonal length of indentation decreases **(Fig 4)**.

In the present study, the enamel specimens were subjected to a demineralizing cycle of 48 hours, following which the enamel microhardness of all the specimens was in range of 163 – 197 VHN. This is in accordance with the study conducted by Lata S[11], where following the demineralizaling cycle the enamel microhardness decreased to a range of 162- 183 VHN.

In Group A (CPP-ACP) microhardness increased significantly to 284.9 $\pm$ 46.38 (Mean increase of 105.8 VHN, ($P<0.001$)) **(TABLE 2)**. The mean increase in microhardness of enamel was more compared to the study conducted by Lata.S et.al where they observed the increase in microhardness to be 185$\pm$ 30 VHN after 5 days of treatment, since the extent of remineralization of subsurface lesion remineralization achieved using CPP ACP is dose-dependent and increased with increase in the time of exposure and duration[11,12].

Karlinsey et.al[13,14] used fTCP and different concentration of sodium fluoride. In both these studies, the microhardness of enamel increased considerably post treatment. A mean increase of 105.6 ± 5.6 VHN[13] and 106.2 ± 7.4 VHN[14] was found respectively. In the present study, the microhardness of enamel specimens treated with f-TCP + NaF showed a mean increase of 108.2$\pm$ 13.8 VHN, which was significant ($P<0.001$) & was in accordance with the results of the study conducted by Karlinsey **(TABLE 2)**.

At present, there is no study where comparison between the three agents used in the present study was done. However, a few studies have compared the remineralization ability of two agents used in study. Zhang et al.[15] in their study used CPP-ACP and 500ppm NaF to evaluate the change in microhardness of eroded enamel. The CPP-ACP crème increased SMH of the eroded enamel significantly more than 500 ppm NaF solution after 30 days. Turssi CP et.al.[16], conducted a study to compare remineralizing potential CPP ACP and CSP containing paste on acid softened enamel. They compared 4 products containing CPP-ACP, CPP-ACP+Fluoride, CSP and fluoridated dentrifice with control. The results obtained showed that the increase in SMH in CPP-ACP & CSP group did not differ significantly and was higher than control group. Similarly, in the present study, no significant difference was found between Group A (CPP-ACP) and Group C (Novamin). Karlinsey et.al[17] compared MI Paste Plus (contains CPP-ACP), Theramed SOS and fTCP + NaF. After 10 days of pH cycling, the results showed that Theramed SOS was superior to all the agents used, however equivalent to Theramed SOS was fTCP + NaF combination which was superior to CPP ACP.

In the present study, we compared the remineralization potential of CPP – ACP, fTCP + NaF, and Novamin over a period of 15 days. The results showed that the increase in microhardness value was highest in f-TCP+NaF group followed by CPP-ACP and Novamin groups respectively. However, no significant difference in the mean values of microhardness ($P>0.05$) after treatment with the three different agents was observed.

V. Conclusion

In the present study, remineralizing potential of three agents showed no significant difference. In terms of surface microhardness change, all the three agents increased the microhardness. However further research is necessary to compare remineralizing ability in terms of net gain of calcium and phosphate gain by the enamel.

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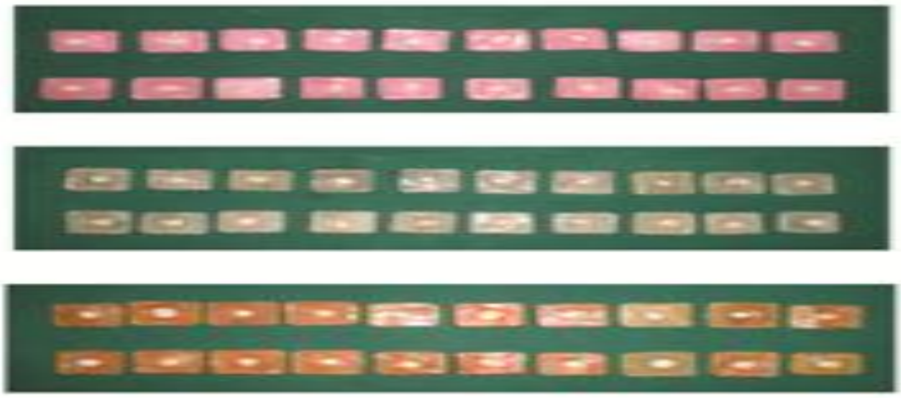

Figure 1: 60 enamel specimens prepared.

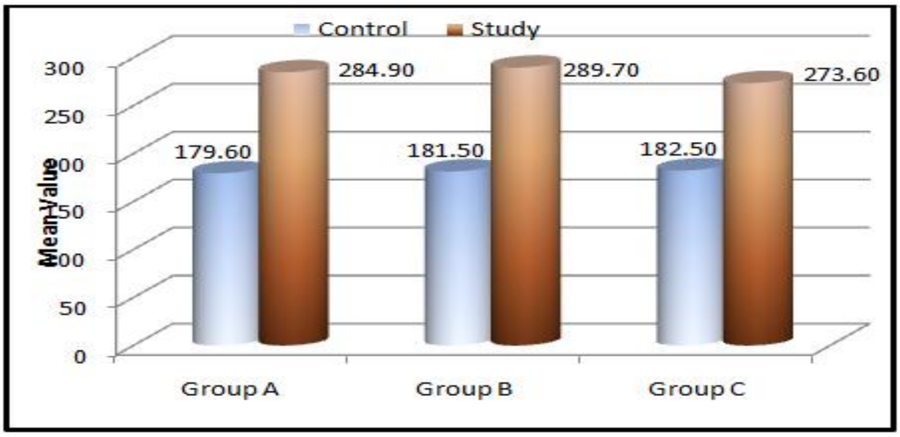

Figure 2: Microhardness values recorded across the three groups

Figure 3: Indentation image after demineralization of enamel specimen

Figure 4: Indentation image after treatment for 15 days**.**