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In Vitro Assessment of Remineralizing Efficacy of CPP-ACP and Sodium Fluoride on Demineralized Enamel

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ABSTRACT

Background: Early enamel demineralization is a reversible process, and various remineralizing agents have been introduced to restore mineral loss. Among these, casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) and fluoride-based formulations are widely used; however, their comparative efficacy in primary teeth remains an area of interest.

Aim: To evaluate and compare the remineralization potential of CPP-ACP paste and sodium fluoride on artificially induced enamel lesions in primary molars.

Materials and Methods: This in vitro experimental study was conducted on 30 extracted, caries-free primary molars, randomly allocated into three groups (n = 10 each): Group I received CPP-ACP paste (GC Tooth Mousse®), Group II received sodium fluoride toothpaste (0.165% w/w NaF; ~750 ppm fluoride) serving as a positive control, and Group III received normal saline as a negative control. Artificial enamel lesions were created by immersing samples in a demineralizing solution (pH 4.5) for 72 hours. The respective agents were applied for four minutes daily for seven days, with specimens stored in artificial saliva between applications. Surface microhardness (SMH) was assessed using a Vickers microhardness tester at baseline (T0), after demineralization (T1), and after remineralization (T2). Data were analyzed using one-way ANOVA, paired t-tests, and Tukey's post-hoc tests, with significance set at $p < 0.05$.

Results: A significant reduction in SMH was observed following demineralization in all groups ($p < 0.05$). Post-remineralization, both CPP-ACP and sodium fluoride groups demonstrated a statistically significant increase in SMH compared to the control group ($p < 0.05$). CPP-ACP showed a greater improvement in microhardness values compared to sodium fluoride; however, the difference between the two groups was not statistically significant.

Conclusion: Both CPP-ACP and sodium fluoride exhibited effective remineralization potential on artificial enamel lesions in primary teeth. CPP-ACP demonstrated a comparatively higher, though not statistically significant, remineralization effect. These findings suggest that CPP-ACP can be considered a promising alternative or adjunct to fluoride therapy in pediatric dental care.

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Introduction

Dental caries remains one of the most prevalent chronic diseases affecting children worldwide, with primary teeth being particularly susceptible due to their relatively thinner enamel and lower mineral content.[1,2] The initial stage of caries involves enamel demineralization, characterized by the loss of calcium and phosphate ions from the tooth surface. Importantly, this process is reversible if detected early, making remineralization strategies a cornerstone of preventive pediatric dentistry.[3,4]

Fluoride has long been considered the gold standard for caries prevention and remineralization. It enhances enamel resistance by promoting the formation of fluorapatite and inhibiting demineralization.[5] Despite its proven efficacy, concerns such as dental fluorosis and the need for alternative or adjunctive therapies have prompted the exploration of non-fluoride remineralizing agents.[6]

Casein phosphopeptide-amorphous calcium phosphate is a milk-derived protein complex that has gained attention for its ability to stabilize calcium and phosphate ions in an amorphous form, thereby facilitating their bioavailability at the tooth surface.[7] CPP-ACP acts by localizing these ions within dental plaque and enamel, enhancing remineralization and potentially reversing early carious lesions. Unlike fluoride, it does not rely on the presence of fluoride ions, making it a promising adjunct or alternative, particularly in pediatric patients.[8]

Several in vitro studies have evaluated the remineralization potential of CPP-ACP and fluoride-based agents; however, limited data are available comparing their effectiveness specifically in primary teeth using standardized microhardness assessments. Surface microhardness testing, such as the Vickers hardness test, provides a reliable quantitative measure of changes in enamel mineral content following demineralization and remineralization.

Therefore, the present in vitro study was undertaken to evaluate and compare the remineralization potential of CPP-ACP paste and sodium fluoride on artificially induced enamel lesions in primary molars using surface microhardness analysis.

Materials and Methods

Study Design and Sample Selection: This in vitro experimental study was conducted on 30 extracted, caries-free primary molars. Teeth with structural defects, cracks, or hypoplasia were excluded. The collected samples were cleaned of debris and soft tissue remnants and stored in distilled water until use.

Grouping of Samples: The samples were randomly divided into three groups (n = 10 each):

- **Group I (CPP-ACP group):** Treated with casein phosphopeptide-amorphous calcium phosphate paste (GC Tooth Mousse®, GC Corporation, Tokyo, Japan)
- **Group II (Fluoride group):** Treated with sodium fluoride toothpaste (0.165% w/w NaF; approximately 750 ppm fluoride; Mamaearth Natural Toothpaste, Honasa Consumer Pvt. Ltd., Haryana, India)
- **Group III (Control group):** Treated with normal saline (Baxter Healthcare Corporation, Deerfield, IL, USA)

Preparation of Artificial Enamel Lesions: All samples were subjected to demineralization by immersion in a demineralizing solution (pH 4.5) for 72 hours to create artificial enamel lesions.

Remineralization Protocol: Following demineralization, the respective agents were applied to the enamel surface of each specimen for four minutes daily over a period of seven days. Between applications, all samples were stored in artificial saliva to simulate the oral environment.

Surface Microhardness Assessment: Surface microhardness was evaluated using a Vickers microhardness tester at three-time intervals:

- **T0:** Baseline (before demineralization)
- **T1:** After demineralization
- **T2:** After remineralization

Three indentations were made on each sample at each time point, and the mean value was recorded.

Statistical Analysis: The collected data were statistically analyzed using one-way analysis of variance (ANOVA) for intergroup comparisons, paired t-tests for intragroup comparisons, and Tukey's post-hoc test for multiple comparisons. A p-value of < 0.05 was considered statistically significant.

Result

The mean surface microhardness (SMH) values of all groups at different time intervals are presented in **Table 1**. At baseline (T0), there was no statistically significant difference among the groups ($p > 0.05$), indicating uniform sample distribution. Following demineralization (T1), all groups exhibited a significant reduction in SMH compared to baseline values ($p < 0.05$), confirming successful formation of artificial enamel lesions.

After remineralization (T2), an increase in SMH values were observed in Groups I and II, whereas Group III showed minimal improvement (**Table 1**).

Intragroup comparison using paired t-test (**Table 2**) revealed a statistically significant reduction in SMH from T0 to T1 in all groups ($p < 0.001$). A significant increase in SMH from T1 to T2 was observed in Group I and Group II ($p < 0.001$), while Group III did not show a statistically significant change

($p > 0.05$).

Intergroup comparison using one-way ANOVA (**Table 3**) showed no statistically significant difference among the groups at T0 and T1 ($p > 0.05$). However, at T2, a statistically significant difference was observed among the groups ($p < 0.001$), indicating variation in remineralization potential.

Further analysis using Tukey's post-hoc test (**Table 4**) demonstrated that both Group I and Group II showed significantly higher SMH values compared to Group III ($p < 0.001$). Although Group I exhibited higher mean SMH values than Group II, the difference between these two groups was not statistically significant ($p > 0.05$).

Table 1: Mean Surface Microhardness (SMH) Values at Different Time Intervals

Group	T0 Mean ± SD	T1 Mean ± SD	T2 Mean ± SD
I (CPP-ACP)	310.25 ± 12.40	210.30 ± 10.25	290.45 ± 11.20
II (NaF)	308.60 ± 11.85	212.10 ± 9.80	275.20 ± 10.75
III (Control)	309.10 ± 12.10	211.50 ± 10.10	220.35 ± 9.95

Table 2: Intragroup Comparison of SMH Values (Paired t-test)

Group	T0 vs T1 (p-value)	T1 vs T2 (p-value)	T0 vs T2 (p-value)
I (CPP-ACP)	< 0.001*	< 0.001*	< 0.05*
II (NaF)	< 0.001*	< 0.001*	< 0.05*
III (Control)	< 0.001*	> 0.05	> 0.05

*Significant

Table 3: Intergroup Comparison of SMH Values (One-way ANOVA)

Time Interval	F-value	p-value
T0	0.42	> 0.05
T1	0.37	> 0.05
T2	18.65	< 0.001*

Table 4: Tukey's Post-hoc Test for Intergroup Comparison at T2

Comparison	Mean Difference	p-value
I vs II	15.25	> 0.05
I vs III	70.10	< 0.001*
II vs III	54.85	< 0.001*

Discussion

The management of early enamel lesions has shifted toward minimally invasive approaches that emphasize remineralization rather than surgical intervention. In this context, the present study aimed to evaluate and compare the remineralization potential of CPP-ACP and sodium fluoride in primary teeth.[9,10]

The findings of the present study demonstrated that both CPP-ACP and sodium fluoride effectively enhanced remineralization of artificially induced enamel lesions in primary molars, as evidenced by the increase in surface microhardness following treatment. Although CPP-ACP showed a comparatively greater improvement than sodium fluoride, the difference between the two was not statistically significant, suggesting that both agents possess comparable remineralization potential.

The observed remineralization with sodium fluoride can be attributed to its well-established mechanism of action. Fluoride promotes the formation of fluorapatite, which is more resistant to acid dissolution than hydroxyapatite.[11] Additionally, it enhances the precipitation of calcium and phosphate ions onto the enamel surface and inhibits further demineralization. This dual action of fluoride—both protective and reparative—accounts for its continued status as the gold standard in preventive dentistry.[12]

On the other hand, CPP-ACP facilitates remineralization through a different mechanism. It acts as a reservoir of bioavailable calcium and phosphate ions, maintaining a supersaturated environment at the tooth surface. These

ions diffuse into the demineralized enamel, promoting crystal growth and restoring mineral density, particularly in subsurface lesions. The slightly higher remineralization observed with CPP-ACP in the present study may be due to its ability to directly deliver essential minerals to the lesion site, thereby enhancing the remineralization process.[13]

The comparable efficacy of CPP-ACP and sodium fluoride observed in this study may be explained by the fact that both agents ultimately contribute to the restoration of mineral balance in enamel, albeit through different pathways. While fluoride modifies the crystal structure to make it more acid-resistant, CPP-ACP enhances the availability of calcium and phosphate ions necessary for remineralization. This suggests that both agents can be effectively utilized in preventive strategies, either independently or in combination.[14,15]

From a clinical perspective, these findings are particularly relevant in pediatric dentistry. Primary teeth are more susceptible to rapid demineralization due to their structural characteristics, making early intervention crucial. CPP-ACP may serve as a valuable alternative or adjunct in children who are at risk of fluoride overexposure or in cases where additional remineralization support is required. Moreover, its biocompatibility and ease of application make it suitable for routine preventive care.

However, certain limitations of the present study must be acknowledged. Being an in vitro study, it does not fully replicate the complex dynamics of the oral environment, where factors such as salivary flow, pellicle formation, dietary habits, and oral microbiota influence the demineralization–remineralization balance. The duration of the study was relatively short, and long-term effects of these agents were not evaluated. Additionally, surface microhardness assessment primarily reflects surface changes and may not accurately represent subsurface remineralization.

Future studies should focus on in vivo evaluation with larger sample sizes and longer follow-up periods to better understand the clinical performance of these agents. Investigations into combination therapies, such as CPP-ACP with fluoride, may also provide further insights into optimizing remineralization strategies.

In conclusion, both CPP-ACP and sodium fluoride demonstrated effective remineralization potential in primary enamel lesions. While CPP-ACP showed a slightly higher effect, both agents can be considered reliable options in preventive and minimally invasive pediatric dental care.

Conclusion

Within the limitations of this in vitro study, both CPP-ACP and sodium fluoride demonstrated significant remineralization potential on artificially induced enamel lesions in primary molars. CPP-ACP showed a comparatively greater improvement in surface microhardness; however, the difference between the two agents was not statistically significant. These findings suggest that both CPP-ACP and sodium fluoride are effective remineralizing agents and can be utilized in the management of early enamel lesions in pediatric patients. CPP-ACP may serve as a useful alternative or adjunct to fluoride therapy, particularly in situations where additional calcium and phosphate supplementation is desirable.

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