RESEARCH ARTICLE

# Comparative Evaluation of Two Remineralizing Agents on Artificial Carious Lesion Using DIAGNOdent

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## Abstract

**Purpose:** The current *in vitro* study was conducted to assess the remineralization potential of CPP-ACP and a customized dentifrice (tricalcium-phosphate) on artificial carious lesions using DIAGNOdent.

**Materials and methods:** Fifty-one extracted premolars that satisfied the inclusion criteria were painted using acid-resistant nail varnish. A window of 4 × 4 mm was exposed on the center of the buccal surface of each tooth. After 20 minutes of drying, the baseline reading of enamel specimens was assessed using DIAGNOdent. The teeth were then immersed in a bath of demineralizing solution. An incubation period of 96 hours at 37°C resulted in artificial caries-like lesions on the specimen. Readings of the specimen within the window after demineralization were recorded using DIAGNOdent for all the samples. The specimens were divided into three groups randomly [group I—casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), group II—customized dentifrice, group III—artificial saliva]. Samples were subjected to the daily treatment regimen for a period of 30 days. The samples were evaluated for remineralization by laser fluorescent device (DIAGNOdent) on the 15th and 30th day, respectively.

**Results:** The statistical analysis was done using the Friedman test, Kruskal–Wallis test, Wilcoxon sign rank test, and Mann–Whitney test. The results showed that both CPP-ACP and customized dentifrice showed almost similar remineralization potential but CPP-ACP showed significant remineralization ($p < 0.001$).

**Conclusion:** The DIAGNOdent observation conclusively proves that CPP-ACP and customized dentifrice remineralizes the demineralized tooth samples *in vitro* with CPP-ACP showing significant remineralization.

**Keywords:** Casein phosphopeptide-amorphous calcium phosphate, Customized dentifrice, DIAGNOdent, Remineralization.

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## Introduction

Dental caries is a chronic disease. It is easily detectable and reversible at an early stage but irreversible and destructive once the cavitation has occurred.[1] Surface lesions make the tooth crippled and lead to irreparable damage.[2]

Accurate and early detection of caries and assessment of lesion progression are important to prevent further damage to the tooth structure.[3]

Recent studies have focused on remineralizing early carious lesion using specific agents, thereby controlling the extent of demineralization.[1]

Demineralization can be defined as the process of mineral loss from tooth structure. And remineralization is the addition of hydroxyapatite crystals to the tooth structure. Remineralization by artificial agents works by creating a supersaturated layer over an early lesion. This prevents the leaching of minerals and forces ions of calcium and phosphate into the voids. These agents usually contain calcium phosphate with or without fluoride.[1]

In the remineralization process, the chemical destruction of enamel is stopped or reversed without the use of restorative material. In children, at low oral pH, the demineralization is higher while at normal oral pH remineralization is lower than in adults.[4]

Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) (Tooth Mousse, GC India) was introduced in 1998 as a remineralizing agent.[1] Various studies have shown CPP-ACP to decrease demineralization and enhance remineralization of the enamel surface carious lesions as well as remineralization of enamel subsurface lesions in various animal and human *in situ* trials.[5]

The customized toothpaste used in the study contains a unique formulation of ingredients including tricalcium-phosphate which other than being easily available are cost-effective as well. It is easy to prepare in laboratory conditions.

Hibst and Paulus discovered that caries has bacterial metabolites that produce fluorescence and can be enhanced by laser light.[6] Based on this DIAGNOdent, a laser cavity detector is the advanced dental technology developed for the detection of tooth decay. When the laser reaches decay the decaying unit emits a fluorescent light, which bounces back to the sensor and is translated into digital units and audible signal. With DIAGNOdent we can know accurately and reliably find decay at an early stage before it causes more widespread damage.[3]

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## Materials and Methods

This is an *in vitro* comparative evaluation study and the study design was approved by the institutional review board. A sample size of 17 teeth per group was used in the study thus totaling 51 subjects in 3 groups based on the statistical analysis.

The three study groups are:

Group I—Experimental group I (GC Tooth Mousse).

Group II—Experimental group II (customized dentifrice).

Group III—Control group (artificial saliva).

All the teeth were cleaned of any remnants and calculus and stored in artificial saliva. The teeth were painted with acid-resistant nail varnish. A window of 4 × 4 mm was created on the center of the buccal surface of each tooth. The nail varnish was then allowed to dry at room temperature and a second coat was applied. After 20 minutes of drying, the baseline reading of enamel specimens was assessed using DIAGNOdent. The teeth were immersed in a bath of the demineralizing solution, incubated at 37°C for 96 hours resulting in the artificial caries-like lesion. Readings of the specimen within the window after demineralization were taken using DIAGNOdent for all the samples.

Composition of the demineralizing solution:

- 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 to 4.52 at 37°C with 50% NaOH.

Remineralization of samples:

Preparation of customized dentifrice

| *Constituents* | *Composition* |
|---|---|
| Tricalcium phosphate | 21 g |
| Bentonite | 750 mg |
| Sodium lauryl sulfate | 125 mg |
| Saccharin sodium | 125 mg |
| Methyl salicylate | 0.125 mL (4 drops) |
| Peppermint oil | 0.12 mL (4 drops) |
| Mucilage of acacia | 3.5 mL |
| Glycerin | 7.5 mL |

The dentifrice is prepared based on the protocol followed by the Department of Pharmacology of Yenepoya Medical College, Mangaluru. The required quantity of bentonite, sodium lauryl sulfate, and saccharin sodium was taken and mixed well in a mortar. To this mucilage of acacia and glycerin was mixed thoroughly. Then, tricalcium phosphate was added to it and mixed well. To this mix, four drops each of methyl salicylate and peppermint oil was added and mixed to form the paste.

### First Cycle

After demineralization, the specimens were divided into three groups randomly (group I, group II, group III). The agents selected for each of the specific groups were applied using cotton applicator tips for 3 minutes, twice daily for 15 days. The samples were then washed under running tap water, stored in artificial saliva for and readings were obtained with DIAGNOdent.

Group III specimen, stored in artificial saliva was considered as the control group and the artificial saliva was changed daily.

### Second Cycle

Pastes were applied for 15 more days, as per the protocol mentioned for the first cycle, and stored in artificial saliva. At the end of 30 days, the samples were again evaluated using DIAGNOdent.

## Results

Data were statistically analyzed and the parametric tests used were:

- Intragroup comparison assessing the difference at various intervals to check the level of significance using Wilcoxon sign rank test.
- Intergroup comparison at various intervals using Kruskal–Wallis test.
- Intergroup comparison of variables at different time intervals using Mann–Whitney test.

When a pairwise comparison of time intervals in each group was done significant difference in DIAGNOdent values were observed except in group III when compared between values at 15 days of remineralization with demineralization, 30 days of remineralization with demineralization, and 30 days of remineralization with 15 days of remineralization (Table 1).

According to statistical analysis when an intergroup comparison of DIAGNOdent values at various time intervals was done (Table 2).

- At baseline, there was no significant difference in DIAGNOdent values with a $p$ value of 0.82.
- After demineralization, there was no significant difference in DIAGNOdent values with a $p$ value of 0.29.
- At 15 days of remineralization, there was a significant difference in DIAGNOdent values in three groups with a $p$ value $< 0.001$.
- At 30 days of remineralization, there was a significant difference in DIAGNOdent values in three groups with a $p$ value $< 0.001$.
- When the group I and group II was compared, there was no significant difference in DIAGNOdent values at any time intervals (Table 3).
- When both the study groups (group I and II) were compared with the control group (group III), there was a significant difference in the DIAGNOdent values on the 15th and 30th day of the remineralization procedure (Table 3).

## Discussion

The process of caries formation is a cycle of remineralization and demineralization with different stages being either reversible or irreversible. The key to dental caries prevention is the balance between remineralization and demineralization. The past few decades have brought in an era of prevention, focusing on the "minimally invasive" approach that emphasizes the detection of these lesions sooner.[7] Researchers have investigated the use of calcium and phosphorous, low cariogenic potential, and possible cariostatic activity of dairy products such as milk, casein, caseinates, and cheese to prevent and/or reverse white spot lesions have shown promising results in various studies. Many remineralization agents work on the principle of ionic exchange mechanism.[8]

In our study, a period of 30 days was allotted for pH cycling, to provide sufficient time for the agents to act on the demineralized enamel specimen. This model involved exposing the enamel specimens for 96 hours of demineralization and acetic acid buffer at a pH of 5.5. Demineralization of enamel leads to the dissolution of HA crystals and diffusion of Ca/P ions onto the enamel surface.

**Table 1:** Intragroup comparison assessing the difference at various intervals to check the level of significance using Wilcoxon sign rank test

| | *Group I* | | *Group II* | | *Group III* | |
|---|---|---|---|---|---|---|
| | *Z* | *p value* | *Z* | *p value* | *Z* | *p value* |
| Demineralization—Baseline | −3.65 | <0.001* | −3.7 | <0.001* | −3.65 | <0.001* |
| 15 days—Baseline | −3.65 | <0.001* | −3.67 | <0.001* | −3.66 | <0.001* |
| 30 days—Baseline | −3.65 | <0.001* | −3.66 | <0.001* | −3.65 | <0.001* |
| 15 days—Demineralization | −3.54 | <0.001* | −3.56 | <0.001* | −1 | 0.32 (NS) |
| 30 days—Demineralization | −3.64 | <0.001* | −3.66 | <0.001* | −1.41 | 0.16 (NS) |
| 30 days—15 days | −3.43 | 0.001* | −3.45 | 0.001* | −1 | 0.32 (NS) |

*$p < 0.05$ statistically significant, $p > 0.05$ non-significant, NS

**Table 2:** Intergroup comparison at various intervals using Kruskal–Wallis test

| | | | | | | *Kruskal–Wallis test* | |
|---|---|---|---|---|---|---|---|
| | *Group* | *N* | *Mean* | *SD* | *Min* | *Chi-square value* | *p value* |
| Baseline | Group I | 17 | 3.53 (1.13) | 1–5 | 4 (3–4) | 0.40 | 0.82 (NS) |
| | Group II | 17 | 3.59 (1.06) | 2–5 | 4 (3–4.5) | | |
| | Group III | 17 | 3.41 (0.71) | 2–4 | 4 (3–4) | | |
| Demineralization | Group I | 17 | 14.35 (2.09) | 8–16 | 15 (14–16) | 2.47 | 0.29 (NS) |
| | Group II | 17 | 13.65 (2.18) | 7–16 | 14 (13.5–15) | | |
| | Group III | 17 | 13.59 (2.72) | 6–16 | 14 (13.5–15) | | |
| 15 days | Group I | 17 | 11.53 (1.46) | 7–13 | 12 (11–12.5) | 17.75 | <0.001* |
| | Group II | 17 | 11.29 (1.80) | 6–13 | 12 (11–12) | | |
| | Group III | 17 | 13.53 (2.70) | 6–16 | 14 (13.5–15) | | |
| 30 days | Group I | 17 | 8.47 (1.28) | 7–11 | 8 (7.5–9) | 22.41 | <0.001* |
| | Group II | 17 | 8.94 (1.35) | 6–12 | 9 (8–9.5) | | |
| | Group III | 17 | 13.47 (2.74) | 6–16 | 14 (13.5–15) | | |

*$p < 0.05$ statistically significant, $p > 0.05$ non-significant, NS

**Table 3:** Intergroup comparison of variables at different time intervals using Mann–Whitney *U* test

| | | *Baseline* | *Demineralization* | *15 days* | *30 days* |
|---|---|---|---|---|---|
| Group I vs group II | U Statistic | 143.50 | 102.00 | 138.50 | 108.00 |
| | *p* value | 0.97 (NS) | 0.13 (NS) | 0.83 (NS) | 0.19 (NS) |
| Group I vs group III | U Statistic | 128.50 | 113.50 | 42.00 | 28.00 |
| | *p* value | 0.55 (NS) | 0.27 (NS) | <0.001* | <0.001* |
| Group II vs group III | U Statistic | 130.50 | 133.00 | 40.00 | 31.50 |
| | *p* value | 0.61 (NS) | 0.68 (NS) | <0.001* | <0.001* |

*$p < 0.05$ statistically significant, $p > 0.05$ non-significant, NS

In this study, a buffered acidic solution was used to produce an artificial enamel subsurface lesion. After demineralization, specimens were subjected to remineralization for a period of 30 days. The samples were evaluated for demineralization and remineralization using a fluorescent technique with a DIAGNOdent.

As recommended by the manufacturer, the cut-off points to indicate the process of demineralization were considered, which indicated that the samples were demineralized. The digital values indicating the demineralized state of the samples were also in agreement with the observations. The samples subjected to remineralization were evaluated at 15 days and 30 days, respectively.

The mean values were significantly lower when compared with readings after demineralization in experimental group I (GC Tooth Mousse) and group II (customized dentifrice) at the end of the test period which was statistically significant. Hence, it was interpreted that the samples of groups I and II showed remineralization with a decrease in DIAGNOdent value which was statistically significant.

Kalra et al. suggested that initial non-cavitated lesions can be remineralized using non-fluoride strategies so that the health of oral tissues is re-established without the concern of fluoride toxicity if ingested at a high level, especially in children.[9] Goswami stated that casein phosphopeptide-based technology is a strong and ideal non-fluoridated remineralizing agent.[10]

Casein, a major milk protein, exists in micelles that stabilize calcium and phosphate ions. Casein, through partial enzyme digestion, releases casein phosphopeptides in sequences which then stabilizes calcium and phosphate ions. The ability of casein to stabilize calcium and phosphate ions resides in sequences that can be released as small peptides (casein phosphopeptides) by partial enzyme digestion. This leads to the development of remineralization technology based on casein phosphopeptide–stabilized amorphous calcium phosphate complexes and casein phosphopeptide stabilized amorphous calcium fluoride phosphate complexes. Various commercial products such as sugar-free

chewing gums, mouthwashes, and dental creams have also incorporated these complexes in the ingredients.[11]

The customized dentrifice in our study was a non-fluoridated toothpaste with the main ingredient being tricalcium phosphate, which when activated by various organic molecules leads to remineralization.

This can be termed a "smart" calcium phosphate system as there is a highly controlled delivery of calcium and phosphate ions to the teeth, with functionalized tricalcium phosphate system. This system also avoids the undesirable reactions of fluoride in storage.[12] It is specially prepared so that it may co-exist with fluoride in aqueous as well as non-aqueous forms, and it is a partially soluble hydroxyapatite precursor. The milling of TCP is done with simple organic materials resulting in a functionalized TCP ingredient that can be used in any particular fluoride vehicle such as denitrifies or varnishes. This process ensures that calcium and fluoride remain active before use, avoiding premature interactions with each other.[13,14] Karlinsey and Mackey in their study states that salivary moisture from the tooth breaks the protective barriers in TCP, rendering the calcium, phosphate, and fluoride ions available to teeth surfaces.[12]

In this study, the efficacy of two remineralizing agents on artificial carious lesions was detected using DIAGNOdent. This was done by recording the changes in the fluorescence of the enamel.

In this study, both CPP-ACP (Tooth Mousse™) and tricalcium phosphate (customized dentifrice) groups could produce remineralization of artificial carious lesions with CPP-ACP being the gold standard and safe non fluoridated remineralizing agent which can be used in children.

## Conclusion

In the current study, it was observed that remineralization of artificial carious lesions was observed with both CPP-ACP (Tooth Mousse™) and tricalcium phosphate (customized dentifrice) groups. Though the remineralization produced by customized dentifrice was similar to CPP-ACP, the CPP-ACP remains the gold standard non-fluoridated remineralizing agent which can be safely used in children.

Further *in vivo* studies have to be done to check the efficacy of customized dentifrice on remineralization of incipient carious lesions.

If the results of the studies are promising a non-fluoridated dentifrice that will be easily available and cost-effective can be used as an alternative to other dentifrices available in the market.

## Clinical Significance

Casein phosphopeptide-amorphous calcium phosphate can be used as a non-invasive method to prevent demineralization and promote remineralization of early enamel lesions. The customized dentifrice has the potential to prevent demineralization and enhance remineralization, thereby arresting the carious lesion at an early stage. Further *in vivo* studies should be done to check the efficacy of customized dentifrice on remineralization of incipient carious lesions. If the study results are promising, the customized dentifrice can be used as an alternative to other dentifrices. A low-cost remineralizing agent can be developed. Results can be contributed to the scientific literature.

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