THE EFFECT OF CASEIN PHOSPHOPEPTIDE- AMORPHOUS CALCIUM PHOSPHATE FLUORIDE, ORGANIC AND INORGANIC FLUORIDE IN PREVENTION OF DENTIN EROSION- AN IN VITRO STUDY.

Dr. Abhidnya P. Gaikwad*, Dr. Meenal Gulve, Dr. Roshan Samuel, Dr. Swapnil Kolhe, Dr. Gayatri Aher, Dr. Kedar Apte

Corresponding Author: *Dr. Abhidnya P. Gaikwad, Address: 4, Nirmala residency, near ushakiransoc, Trimbakrd, Nashik-422002, Email- abhidnyagaikwad1116@gmail.com

ABSTRACT
Context: Dental erosion is defined as the loss of tooth substance by acid exposure not involving bacteria. To enhance the remineralization and to prevent further progression of dental wear, highly-concentrated fluoride applications and calcium rich compounds are recommended.

Aims: The purpose of this study is to evaluate effect of casein phosphopeptide-amorphous calcium phosphate fluoride paste, organic and inorganic fluoride on the prevention of dental erosion.

Methods and Design: In vitro Randomised Control trial

Methods and Material: This in vitro experimental study 80 sound human premolar teeth (with no caries or fracture) were included. Buccal surfaces of the teeth were ground flat and polished with abrasive discs. Afterwards, half the tooth surfaces were covered with adhesive tape to maintain reference surface and samples were then randomly divided into four groups of 20 each. Samples were randomly allocated into four groups. Group A was pretreated with GC tooth mousse plus 4 times a day for 5 days. Group B was pretreated with 0.2% sodium fluoride mouthwash 4 times a day for 5 days. Group C was pretreated with amine fluoride mouthwash 4 times a day for 5 days. Group D was considered as the control group with no pretreatment. In the next step, the samples were exposed to Coca-Cola 4 times a day for 3 days. After each erosive cycle, the samples were rinsed with deionized water and stored in artificial saliva. The surface loss was determined using profilometry.

Statistical analysis used: Unpaired ‘t’ test and ANOVA test.

Results: The erosion in both Groups A, B and C was less than the control group. The surface loss in GC tooth mousse plus and mouthwash group was significantly lower than in the control group. Erosion in GC tooth mousse plus group was more than the mouthwash group and less than the control group.

Conclusions: Amine fluoride mouthwash is more effective for prevention of dentin erosion.

Key-words: Amine fluoride, casein phosphopeptide-amorphous calcium phosphate- fluoride; dentin; erosion; sodium fluoride

INTRODUCTION
• Dental erosion is a complex phenomenon that involves a localized mineral loss from tooth surfaces, without the involvement of microorganisms.
Several published studies have shown an increasing prevalence of enamel erosion among young patients, and the relationship between dental erosion and excessive consumption of acidic foods mainly soft drinks has been reported.\textsuperscript{1} One of the ways proposed for diminishing this deleterious effect of soft drinks on tooth erosion is by modifying their composition in order to reduce their demineralizing power.\textsuperscript{2,3} However, the effectiveness of product modification will depend on many factors, including concentration and solubility of the additives, complex formation, position of the equilibrium point, pH, and temperature.\textsuperscript{2} Another strategy for protecting the enamel against acid erosion is the use of solutions and dentifrices with different concentrations of fluorides.\textsuperscript{4-6} Various studies have evaluated the role of fluoride mainly sodium fluoride, sodium monofluorophosphate which are inorganic in nature in the prevention and reduction of erosive wear.\textsuperscript{7} It is also found that organic fluoride like amino fluoride reduce the solubility of the enamel.\textsuperscript{1} Now-a-days, toothpastes or topical creams containing casein phosphopeptide-amorphous calcium phosphate fluoride (CPP-ACPF) are available to counterattack the effects of dental erosion.\textsuperscript{8} Until date, no study has compared the efficacy of CPP-ACPF Paste, organic and inorganic fluoride on prevention of erosive wear. In this study, profilometry was used for measurement of erosion on tooth surfaces.

MATERIALS AND METHODS

This in vitro experimental study was conducted on 80 sound human premolar teeth (with no caries or fracture) that had been extracted as the result of periodontal disease or for orthodontic treatment. Samples used in the study are shown in Fig 1. Materials used in the study are shown in Fig 2.

PREPARATION OF SAMPLES (Fig 3)

- Buccal surfaces of the teeth were ground flat and polished with silicone discs.
- Afterwards, half the tooth surfaces were covered with adhesive tape and samples were then randomly divided into four groups of 20 each.
- Three clean and transparent glasses were prepared and a circle was drawn on their internal surfaces in a height higher than the mid-height of the longest tooth.
- The drawn circle was covered with adhesive tape.
- In the next step, the samples were glued to the external surface of glasses (each group on one glass) in a way that the covered halves of the teeth were placed above the drawn circle and their remaining halves were located below it.
- In the next steps, these glasses were placed in dishes containing acid or mouthwash.
- The amount of acid and mouthwash was regulated in a way in alignment with the drawn circle.
- By doing so, we made sure that the upper halves of teeth were not exposed to acid or mouthwash and were protected.

PRETREATMENT

- Three groups received pretreatment before placing in acid solution.
- GROUP A: Samples received pretreatment with CPPACP-F paste. CPPACP-F paste was applied on samples 4 times a day (5 min each time) for 5 days.
GROUP B: Samples were pretreated with 0.2% sodium fluoride mouthwash. Samples were placed in the mouthwash 4 times a day (1 min each time) for 5 days.

GROUP C: Samples were pretreated with Amine fluoride mouthwash. Samples were placed in the mouthwash 4 times a day (1 min each time) for 5 days.

After each phase of pretreatment, the teeth were irrigated with deionized water and stored in artificial saliva.

GROUP D: (controls) received no pretreatment and stored in artificial saliva.

EXPOSING TEETH TO ACID

The samples were placed in a glass of Coca-Cola 4 times a day (2 min each time) for 3 days.

After each phase, samples were rinsed with deionized water and stored in artificial saliva until the next phase of the test.

PROFILOMETRY (Fig 4)

In the next phase, the amount of surface loss in samples was measured by a profilometer.

For each tooth, profilometry was performed 3 times (for the protected surface, for the eroded surface and for a total surface area)

Finally, the protected and eroded surfaces in each tooth were compared with each other. The eroded surfaces of the four groups were also compared.

RESULTS

To compare the mean surface roughness for eroded and protected surfaces unpaired ‘t’ test is applied at 95% confidence level and 38 degree of freedom separately for each group. (Table no: 1)

It can be observed that there is no significant difference between the mean surface roughness of the teeth in Group A (GC Tooth mousse plus) and Group C (amine fluoride mouthwash), while Group D (control group) and Group A (sodium fluoride mouthwash)

In case of control eroded mean surface roughness was found highest while it was lowest in case of Group C (amine fluoride mouthwash).

For protected group, mean surface roughness was found highest in control and lowest in Group B (sodium fluoride mouthwash)

Comparison of Eroded groups was done using ANOVA at 95% confidence level and 3 and 76 degree of freedom. (Table no: 2) to find if there is any significant difference.

From table no : 2 it can be observed that except group A and C all other pairs show significant difference in mean surface roughness .

If all the mean surface roughness are compared Group C (amine fluoride mouthwash), shows least roughness while highest in Group D (control group)

DISCUSSION

Erosion is chemical tooth wear resulting from acids in foods and beverages.

Considering the growing consumption of soft drinks, the present study was conducted aiming at evaluating the role of preventive factors in prevention of erosion due to the consumption of Coca-Cola which is a popular drink worldwide. ¹

Application of ionized fluoride, i.e., sodium fluoride, amine fluoride or stannous fluoride on tooth surfaces results in deposition of calcium fluoride (CaF₂) on the enamel surface. Under neutral conditions, this layer can stay for weeks, even months on tooth surfaces. Proteins and phosphate ions can also incorporate into the structure of this deposit layer. CaF₂ deposition is facilitated by increasing the concentration of
fluoride ion, longer exposure time, and lowering the pH of the solution. CaF₂ deposition has been observed as deep as 40μ in dentin. The fluoride ions released from CaF₂ can incorporate into the dental hard tissue and result in its further stability, hardness and increased abrasion resistance. When enamel and dentin are exposed to fluoride ions, the calcium and phosphate present in tooth structure form fluorapatite crystals with these ions. This compound is more acid resistant than hydroxyapatite.9

- Recent laboratory studies have shown that calcium-containing compounds can prevent dental erosion. CPP-ACP complex provides optimal concentrations of calcium and phosphate ions for enhancement of enamel remineralization.10

- Tooth mousse (TM) is a water-based sugar-free cream that contains CPP-ACP. When applied, it maintains optimal concentrations of calcium and phosphate ions on enamel surfaces to enhance remineralization.11

- The nanocomplex CPP-ACP is a bioactive agent that increases the level of Ca²⁺ and PO₄³⁻ ions in the bacterial biofilm. During an erosive attack, the CPP-ACP could release Ca²⁺ and PO₄³⁻ ions, supersaturating the media with these ions and creating an environment favorable to enamel remineralization.12

- In vitro studies have demonstrated that CPP-ACP can be absorbed by the salivary pellicle and dental plaque. Thus, a calcium-rich reservoir is formed that can facilitate remineralization.10

- In accordance with study conducted by, Reynolds EC, Cai F, Cochrane NJ, Shen P, Walker GD remineralization of enamel was more with CPP-ACPF followed by NaF.13

- Studies conducted have shown that fluoride supplementation in the form of mouthwash increases the concentration of fluoride ion in the mouth which subsequently results in strengthening of teeth surfaces.14

- In 1957, Muhleman et al. found that organic fluoride like amino fluoride compounds were superior to inorganic fluorides in reducing the solubility of the enamel and has also stated that amino fluorides produce the most powerful enrichment in fluoride of the enamel, even in low concentration.15

- The properties of the amine-fluoride, which are the grounds for obtaining these results, are given by Banoczy16 - the fast distribution of fluoride and its concentration on dental surface; this is conferred by a special structure: tensioactive agents. - its tensioactive properties increase the contact time with the dental plaque 4 times. - the increase of the absorption and the forming of a deposit of fluoride on the enamel surface is ensured by its tensioactive properties and also by the acid environment. - the resistance of enamel to acid attack is ensured by the existence of the CaF₂ precipitates. - promoting the remineralization of initial lesions by realizing high quantities of fluoride during the acid attack. - antibacterial properties.

- The results of this study revealed that both CPP-ACPF paste, sodium fluoride and amine fluoride mouthwash were capable of reducing erosion.

- The rate of erosion(surface loss) in the control group was higher than the tooth mouse and mouthwash groups.

- Among the mouthwash group, Amine fluoride mouthwash showed least loss in surface roughness.
• It can be concluded that among all 4 groups, group 3 that is amine fluoride mouthwash is the best option with least surface roughness.

CONCLUSION
• This study showed that using mouthwash is an effective method for prevention of erosion in at-risk patients.
• Amine fluoride mouthwash provides protection against caries and prevents erosive tooth wear by strengthening the enamel surface. This product is cheap and easily available.
• Thus, if recommended, its consumption will be widely accepted by the public.

REFERENCES
16) Banoczy et al. Effect of amine fluoride/ stannous fluoride containing toothpaste and

Fig 1 Samples used in the study
Fig 2: Material used in the study
Fig 3: Sample preparation

Fig 4: Sample analysis under profilometer
Table no 1: Individual comparison of Eroded and Protected of each group.

<table>
<thead>
<tr>
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<th>GROUP A</th>
<th>GROUP B</th>
<th>GROUP C</th>
<th>GROUP D</th>
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<tr>
<td>Protected surface</td>
<td>0.4455</td>
<td>0.2712</td>
<td>0.5459</td>
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<td>Eroded Surface</td>
<td>0.517</td>
<td>0.6931</td>
<td>0.4828</td>
<td>4.5917</td>
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<tr>
<td>Combined area</td>
<td>1.3421</td>
<td>1.5123</td>
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<td>SURFACE LOSS</td>
<td>0.8966</td>
<td>1.2411</td>
<td>0.7673</td>
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<tr>
<td>P-value</td>
<td>0.138</td>
<td>0.000</td>
<td>0.256</td>
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<tr>
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Table no 2: Comparison of Eroded Groups

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<tr>
<td>A and C</td>
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<tr>
<td>B and D</td>
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<tr>
<td>C and D</td>
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