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# Effect of various remineralizing agents on eroded enamel –an in vitro study

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# ABSTRACT

AIM: To assess the effect of CPP-ACP (Tooth mousse,GC INDIA) , CPP-ACPF (Tooth mousse plus,GC INDIA) and Arginine containing paste (Sensitive pro relief ,COLGATE INDIA) remineralizing agents on enamel surface after erosive challenge. MATERIALS AND METHODS: Buccal and lingual enamel surfaces of the selected 20 molar teeth were used, and embedded in acrylic resin and enamel surfaces were ground flat with Silicon carbide paper disc. Samples were assigned into 4 groups with 10 samples each. Group1 was the control with no treatment, Group 2 of CPP-ACP, Tooth mousse, GC INDIA, Group 3 of CPP-ACPF, Tooth mousse plus, GC INDIA and Group 4 of Arginine containing paste( Sensitive pro relief, COLGATE INDIA). The specimens were then exposed to erosive challenge and treatment. The surface roughness's of the samples were evaluated before and after erosive cycles using surface contact profilometer.

RESULTS: There was no significant difference seen while comparing before and after values for the groups. In intragroup comparison, significant difference for RA before and RA after values in all 4 groups were seen.

**Key words:** Casein phosphopeptide-amorphous calcium phosphate–fluoride–dentin– erosion–arginine containing paste

## **1** INTRODUCTION

Dental erosion is defined as loss of tooth substance by chemical processes not involving bacteria and caused by a variety of extrinsic and intrinsic factors.<sup>1</sup> By changing lifestyle during the recent decades, consumption of acidic foods and beverages has increased. Role of food acids as the main cause of erosion has been documented in numerous studies. Dentin hypersensitivity is usually associated with cervical erosion and has been suggested as a direct clinical outcome

#### of $erosion.^2$

Changes in dietary and oral hygiene habits, oral products, and toothpastes must be made to prevent or decrease the progression of erosion<sup>3</sup>. e.g. the dentifrice containing 8.0% arginine and calcium carbonate. Conventional fluorides, such as sodium fluoride (NaF) and amine fluoride (AmF), form a calcium-rich (CaF2) layer on the tooth surface, which may then provide a physical barrier and a mineral reservoir promoting remineralization and thus modify the erosive process.<sup>4,5</sup>

It has been demonstrated that the application of high concentrations of fluoride increases abrasion resistance and decreases the development of tooth enamel erosion

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in some cases<sup>6-9</sup>. 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.<sup>2</sup>

GC 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.<sup>10</sup> The Nano complex CPP-ACP is a bioactive agent that increases the level of Ca2+ and PO4<sup>3-</sup> ions in the bacterial biofilm. During an erosive attack, the CPP-ACP could release Ca2+ and PO4 <sup>3-</sup> ions, supersaturating the media with these ions and creating an environment favorable to enamel remineralization.<sup>11</sup>

Since there are fewer studies comparing the effects of CCP-ACP, CCP-ACPF and arginine containing dentifrices; our study aims to assess the effect of Casein phosphopeptide-Amorphous calcium phosphate (CPP-ACP, Tooth mousse, GC INDIA), Casein phosphopeptide-Amorphous calcium phosphate with fluoride (CPP-ACPF, Tooth mousse plus, GC INDIA) Arginine containing paste (Sensitive pro relief, COLGATE INDIA) remineralizing agents on enamel surface after erosive challenge.

# 2 MATERIALS AND METHODS

Twenty freshly extracted human molars with intact tooth structure were included. Teeth that were carious or vertically fractured were excluded.

# SPECIMEN PREPARATION:

20 freshly extracted human molars were selected for the study (n=10 for each group). 40 Enamel specimens ( $4 \times 4 \times 2$  mm) were prepared from the buccal and lingual surfaces of the selected teeth, using double faced diamond disc mounted on contra-angle handpiece and embedded in acrylic resin and enamel surfaces will be ground flat with SiC paper disc.

Samples were randomly assigned into 4 groups. Group1: control group where no treatment was given, Group 2: Casein phosphopeptide-Amorphous calcium phosphate (CPP-ACP, Tooth mousse,GC INDIA, Group 3: Casein phosphopeptide-Amorphous calcium phosphate with fluoride (CPP-ACPF, Tooth mousse plus, GC INDIA) and Group 4: of Arginine containing paste (Sensitive pro relief, COLGATE INDIA).

# EROSIVE CHALLENGE AND TREATMENT:

The enamel specimens were exposed to remineralizing agents before each erosive challenge. Specimens were immersed in 2% citric acid using separate containers, at room temperature, for 5 minutes 4 times per day. The specimens were rinsed thoroughly with the deionized water and immersed in artificial saliva at room temperature, between erosive challenges and overnight. This erosive challenge was repeated for 5 days. The 2% citric acid and artificial saliva was changed after every cycle.

#### ROUGHNESS MEASUREMENT:

The surface roughness's of the samples were evaluated before and after erosive cycles using surface contact profilometer. Each sample was scanned with a diamond stylus across the surface under constant load and computes the numeric values representing the roughness of the profile as Ra. The Ra value described the overall roughness of a surface and is defined as the arithmetic mean value of all absolute distances of the roughness profile.

## STATISTICAL ANALYSIS:

All analyses were performed using version 21.0 of the Statistical Package for Social Sciences (IBM Corporation, Armonk, New York, USA. Mean, standard deviation (SD), minimum and maximum values for RA before and after in different groups were calculated. Shapiro-Wilk test showed that RA before and after values did not follow normal distribution curve. Hence, non-parametric tests were used for further data analysis. For comparison between different groups for RA before and after values Kruskal Wallis test was applied. When Kruskal Wallis test showed significant difference between the groups, Mann Whitney U test was used for pair wise comparison.For Intra group comparison of RA before and after values Wilcoxon Signed Ranks test was applied. P values <0.05 were accepted as statistically significant.

## 3 **RESULTS**

Table 1 shows comparison between the groups for before and after RA values. P = 0.897 (>0.05) for RA before values. There was no significant difference between the groups. , P = 0.000 (0.001) Significant difference groups were not same for RA after values .Group 3=Group4< Group2< Group1. Table 2 shows comparison of before and after values in each group. significant difference for RA before and RA after values in all 4 groups were seen. For group 1; RA after value was significantly higher than RA before values. For groups 2, 3 and 4; RA before value was significantly higher than RA after values. (Table 1)

#### Table 1.

×	Groups	N·¤	Mean∙¤	Std. Deviation	Minimum·¤	Maximum∙¤	Kruskal-wallis-test-¤
Ra_before-¤	1.¤	10-¤	1.40 ≔	0.64.≅	0.84∙≍	3.03-¤	χ <sup>2</sup> ·=·0.596,··P·=·0.897
	2.≍	10 ≈	1.43 🛪	0.52·¤	0.2 <b>9</b> ·¤	2.16-#	(>0.05) Not Significant Groups-were-same-for RA-before-values. No
	3-¤	10-¤	1.34∙¤	0.57 ⊭	0.18.≍	2.01-¤	
	4.≍	10-¤	1.44 ≅	0.80 ≔	0.25∙≍	2.80-¤	difference∙between-the groups¤
	R						
Ra_after.⇔	1.¤	10-¤	2.44-¤	0.56-¤	1.08-≍	3.01-¤	χ <sup>2</sup> =-24.891,…P·=-0.000 (0.001)· Significant
	2.⊭	10∹¤	0.80 🛱	0.34 ⊭	0.33 ×	1.26 🗷	Difference ¶
	3-¤	10-¤	0.40 ≈	0.31 ⊭	0.04 ≅	0.84 ⊭	Groups-were-not-same for-RA-after-values¶
	4.≍	10-¤	0.37-¤	0.31 ¤	0.01.≭	0.85-¤	Gr·3=Gr4<·Gr2<·Gr1*
	8						

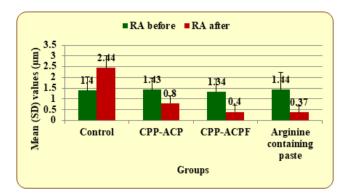


Figure 2. Comparison of RA before and RA after values between different groups

# 4 DISCUSSION

Erosion is chemical tooth wear resulting from acids in foods and beverages. Role of acids in tooth erosion has recently come into the spotlight. Dentin hypersensitivity is among the direct outcomes of erosion that may occur in clean tooth surfaces. Acid reflux and acidic foods and beverages can dissolve the smear layer and expose dentinal tubules to the oral cavity resulting in aggravation of dentin hypersensitivity.<sup>12</sup>The erosion could be prevented by high calcium concentration and possibly phosphate concentration. CPP-ACP has been well documented for re-hardening the softened enamel. ACP located at enamel surface probably buffered the free calcium and phosphate ion activities, causing the supersaturation of ions which depressed demineralization and enhanced remineralization. From another point, the treatment of CPP-ACP facilitated the formation of a crystal layer, filling the interprism, and partially covering the prisms, therefore preventing acid attack.<sup>13</sup>

This study showed CPP-ACP, CPP-ACPf and arginine containing pastes effective against erosion. Casein phosphopeptide amorphous calcium phosphate (CPP-ACP) nanocomplexes are casein-derived peptides in which ACP is stabilized by CPP, and these nanocomplexes act as a calcium and phosphate reservoir when incorporated into the dental plaque and on the tooth surface. The remineralization effect of CPP-ACPf was found to be superior to that of CPP- ACP alone. It is likely that a combination of CPP-ACP and fluoride resulted in co-localization of calcium and phosphate ions with fluoride ions at the enamel surface, presumably as CPP-ACPP nanocomplexes.<sup>14</sup>

Jayaranjan et al. they concluded that that because of the added benefit of fluoride (NaF 0.2%), CPP-ACPF (Tooth Mousse-Plus) showed marginally more amount of remineralization than CPP-ACP (Tooth Mousse).<sup>15</sup>

Poggio et al studied the evaluation of a CPP-ACP paste and of a desensitizing toothpaste (Colgate Sensitive Pro Relief, Colgate Palmolive) on preventing enamel erosion produced by a soft drink (Coca Cola) by using Atomic Force Microscopy (AFM). They concluded that the use of new formulation toothpastes: a CPP-ACP paste (GC Tooth Mousse, GC Corp.) and a desensitizing toothpaste (Colgate Sensitive Pro Relief, Colgate-Palmolive) prevents enamel erosion produced by a soft drink (Coca Cola)<sup>16</sup>

Colgate Sensitive Pro Relief was developed through the association of arginine, an amino acid that is positively charged at physiological pH, pH 6.5-7.4. Sensitive Pro Relief prevents erosive surface loss due to the possible effects of synergistic action between arginine and fluoride. The toothpaste containing arginine was shown to provide more effective protection because the results obtained showed significant difference in the profilometric tests. This fact may be attributed to the association of all components that are present in its formulation.

it is important to emphasize that the results of this study provide indications of what actually happens in the enamel surface. Howe

ver, additional in situ and clinical studies with appropriate designs should be conducted to confirm these in vitro results. Sensitive Pro Relief prevents erosive surface loss due to the possible effects of synergistic action between arginine and fluoride. This dentifrice is a good clinical alternative to reduce dental erosion and sensitivity.<sup>17</sup>

#### 5 CONCLUSION:

Under the conditions of this study, it can be concluded that all the pastes (CPP-ACP, CPP-ACPf, arginine containing paste) tested offer p rotection a gainst e rosion. CPP-ACPf and arginine containing paste showed lesser wear reduction in enamel as compared to CPP-ACP.

# REFERENCES

- [1] Ayad F, Ayad N, Zhang Y, Devizio W, Cummins D, Mateo L. Comparing the efficacy in reducing dentin hypersensitivity of a new toothpaste containing 8.0% arginine, calcium carbonate, and 1450 ppm fluoride to a commercial sensitive toothpaste containing 2% potassium ion: an eight-week clinical study on Canadian adults. J Clin Dent. 2009;20:10–16.
- [2] Srinivasan N, Kavitha M, Loganathan SC. Comparison of the remineralization potential of CPP–ACP and CPP–ACP with 900ppm fluoride on eroded human enamel: An in situ study. Archives of Oral Biology. 2010;55(7):541–544. Available from: https://dx.doi.org/10.1016/j.archoralbio.2010. 05.002.
- Wiegand A, Attin T. Design of Erosion/Abrasion Studies – Insights and Rational Concepts. Caries Research. 2011;45(1):53–59. Available from: https://dx.doi.org/10. 1159/000325946.
- [4] Rees J, Loyn T, Chadwick B. Pronamel and tooth mousse: An initial assessment of erosion prevention in vitro. Journal of Dentistry. 2007;35(4):355–357. Available from: https:// dx.doi.org/10.1016/j.jdent.2006.10.005.
- [5] Yamashita JM, Torres NM, Moura-Grec PG, Marsicano JA, Sales-Peres A, Sales-Peres SHC. Role of arginine and fluoride in the prevention of eroded enamel: anin vitromodel. Australian Dental Journal. 2013;58(4):478–482. Available from: https://dx.doi.org/10.1111/adj.12110.
- [6] Mahoney E, Kilpatrick N. Dental erosion: Part 1. Aetiology and prevalence of dental erosion. N Z Dent J. 2003;99:33–41.
- [7] Bartlett D. Etiology and prevention of acid erosion. Compend Contin Educ Dent. 2009;30:616–636.

- [8] Poggio C, Ceci M, Beltrami R, Lombardini M, Colombo M. Atomic force microscopy study of enamel remineralization. Ann Stomatol (Roma). 2014;5(3):98–102.
- [9] Austin R, Stenhagen KR, Hove LH, Tveit AB, Moazzez R, Bartlett D. The Effect of Single-Application Fluoride Treatment on Simulated Gastric Erosion and Erosion-Abrasion of Enamel In Vitro. The International Journal of Prosthodontics. 2014;27(5):425–426. Available from: https://dx.doi.org/10.11607/ijp.3956.
- [10] Wang CP, Huang SB. The CPP-ACP relieved enamel erosion from a carbonated soft beverage: An in vitro AFM and XRD study. Arch oral biol;2014(5):277–282.
- [11] Magalhães AC, Wiegand A, Rios D, Honório HM, Buzalaf MAR. Insights into preventive measures for dental erosion. Journal of Applied Oral Science. 2009;17(2):75– 86. Available from: https://dx.doi.org/10.1590/s1678-77572009000200002.
- [12] Jayarajan J, Janardhanam P, Jayakumar P, Deepika. Efficacy of CPP-ACP and CPP-ACPF on enamel remineralization - An in vitro study using scanning electron microscope and DIAGNOdent<sup>®</sup>. Indian Journal of Dental Research. 2011;22(1):77–77. Available from: https://dx.doi. org/10.4103/0970-9290.80001.
- [13] Ganss C, Klimek J, Brune V, Schürmann A. Effects of Two Fluoridation Measures on Erosion Progression in Human Enamel and Dentine in situ. Caries Research. 2004;38(6):561–566. Available from: https://dx.doi.org/10. 1159/000080587.
- [14] Moezizadeh M, Alimi A. The effect of casein phosphopeptide-amorphous calcium phosphate paste and sodium fluoride mouthwash on the prevention of dentine erosion: An in vitro study. Journal of Conservative Dentistry. 2014;17(3):244–244. Available from: https://dx.doi. org/10.4103/0972-0707.131787.
- [15] Docimo R, Montesani L, Maturo P. Comparing the efficacy in reducing dentin hypersensitivity of a new toothpaste containing 8.0% arginine, calcium carbonate, and 1450 ppm fluoride to a benchmark commercial desensitizing toothpaste containing 2% potassium ion: an eight-week clinical study in. J Clin Dent. 2009;20:137–143.
- [16] Reynolds EC, Cai F, Cochrane NJ, Shen P, Walker GD, Morgan MV, et al. Fluoride and Casein Phosphopeptide-Amorphous Calcium Phosphate. Journal of Dental Research. 2008;87(4):344–348. Available from: https://dx.doi. org/10.1177/154405910808700420.
- [17] Tezel H, Ergucu Z, Onal B. Effects of topical fluoride agents on artificial enamel lesion formation in vitro. Quintessence Int. 2002;33:347–352.

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