## AN IN VITRO COMPARATIVE EVALUATION OF ENAMEL MICROHARDNESS IN SOFT DRINKS, CPP-ACP, AMINE FLUORIDE AND SODIUM FLUORIDE WITH FUNCTIONALISED TRICALCIUM PHOSPHATE

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#### ABSTRACT

## BACKGROUND

Objectives - Tooth erosion is a common dental problem. It is a localised loss of tooth surface by a chemical process of acidic dissolution of non-bacterial origin. Softening of the enamel surface is an early manifestation of the erosion process. In recent times, the prevalence of dental erosion is steadily increasing. Management of erosion is thus becoming important to the long-term health of the human dentition. In the initial stages, erosion can be reversed by remineralising agents. Several remineralising agents are available in the market. Hence, the aim of the present study was to assess the enamel microhardness of teeth demineralised following exposure to an acidic beverages like carbonated drink - coca cola and evaluating the remineralising potential of recently available remineralising pastes, namely CPP-ACP (GC - Tooth Mousse), Amine fluoride (Amflor) and NaF with functionalised tricalcium phosphate (Clinpro).

### MATERIALS AND METHODS

Thirty extracted human maxillary incisors were decoronated and embedded in self-cured acrylic resin. Baseline microhardness of enamel was measured and samples were randomly divided into 3 main groups with each having two subgroups. After demineralisation with carbonated drink (cola), they were tested for microhardness. For Group I - 0.5 mm layer of CPP-ACP was applied on enamel surface for 3 min and samples were stored in artificial saliva (Group IA) and deionized water (Group IB). For Group II - 0.5 mm layer of amine fluoride was applied for 3 min and samples were stored in artificial saliva (Group IIA) and deionized water (Group IIB). For Group II - 0.5 mm layer of functionalised Tricalcium Phosphate (Clinpro) was applied for 3 min and samples were stored in artificial saliva (Group IIB). All the samples were stored in respective solution for six hours at 37°C. After this microhardness values were evaluated for all the groups and data analysis was done by One-Way ANOVA technique.

#### RESULTS

Among all the groups,

- Samples stored in artificial saliva showed increased microhardness than the samples in deionized water.
- Statistically significant difference was seen between Group IIA (Amflor in artificial saliva) and Group IIB (Amflor in deionized water).
- Group IIA (Amflor in artificial saliva) demonstrated increased microhardness values after remineralisation.

#### CONCLUSION

All the three remineralising agents {CPP-ACP (GC-Tooth Mousse), Amine fluoride (Amflor), NaF with fTCP - (Clinpro)} are effective in reversing the effects of tooth erosion. Their remineralising potential is comparable. All these pastes demonstrated superior results in artificial saliva as opposed to deionized water.

#### KEYWORDS

Artificial Saliva, Amine Fluoride, Carbonated Drink (Cola), Casein Phosphopeptide-Amorphous Calcium Phosphate, Dental Enamel, Sodium Fluoride with Functionalised Tricalcium Phosphate, Tooth Erosion, Vickers Hardness Tests.

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Financial or Other Competing Interest: None	BACKGROUND
Submission 07-12-2016, Peer Review 01-01-2017,	Dental erosion is a contemporary disease, mostly due to the
Acceptance 06-01-2017, Published 12-01-2017.	change in dietary patterns in modern society. It is a "silent"
Corresponding Author:	and multifactorial disease, highly influenced by habits and
Dr. Dilip Katakam,	lifestyles Frecien is a localised loss of tooth surface by a
C/o. Sai Dental Hospital,	inestyles. Erosion is a localised loss of tooth surface by a
Opposite Homeopathy College,	chemical process of acidic dissolution of non-bacterial
Eluru Road,	origin. <sup>1</sup> It is primarily attributed to the ingestion of organic
Gudivada-521301.	and inorganic acidic substances. Tooth erosion may be
E-mail: dilipkatakam@gmail.com	extrinsic or intrinsic Excessive consumption of acidic food
<u>D0I: 10.14260/jemds/2017/62</u>	and have a set and of the most some on article for
	and beverages are one of the most common extrinsic factors
BY NC ND	causing dental erosion. <sup>2</sup>

Intrinsic factors include Gastro-Oesophageal Reflux Disease (GERD), anorexia, bulimia, chronic alcoholism or gastrointestinal disorders.<sup>3</sup>

Among various approaches to treat tooth erosion, preventive measure is an important approach which enhances the acid resistance to the tooth structure and promotes the process of remineralisation. Remineralisation can be achieved naturally from the oral environment (Saliva) or artificially through the application of remineralising agents. Saliva provides protective effects by neutralising and clearing the acid and it is also a good source of inorganic ions calcium phosphate necessary like and for the remineralisation process.<sup>4</sup> Studies have reported that enamel softened by acidic beverages were rehardened following exposure to saliva or artificial saliva.5,6

Fluoride present in saliva also plays a significant role in shifting the equilibrium towards remineralisation. It is a wellknown fact that fluoride enhances the remineralisation of initial carious lesions<sup>7</sup> and early erosion lesions by absorbing partially dissolved crystal lattice the onto of hydroxyapatite.<sup>2,8</sup> Fluorides can be introduced into the oral environment via personal (Dentifrices, mouth rinses) or professional applications (Varnishes, foams, gels, etc.).9 They are available both in inorganic and organic forms. Inorganic include forms sodium fluoride, sodium monofluorophosphate, stannous fluoride and acidulated phosphate fluoride gel. Amine fluoride is a type of organic fluoride introduced by Muhleman in 1967 with a high bioavailability of fluoride. Amflor (Group Pharmaceuticals) is a popular brand of organic fluoride toothpaste presently available in the market.

Milk and cheese are well known dietary agents for enhancing remineralisation due to their high content of MATERIALS USED IN THE STUDY calcium and phosphate.<sup>10,11</sup> Casein Phosphopeptide Amorphous Calcium Phosphate (CPP-ACP) derived from milk protein has been used as a supplemental source of calcium and phosphate ions in the oral environment. This is commercially available as GC Tooth Mousse (GC Asia Dental), which is a promising agent to promote remineralisation.

Tricalcium phosphate (TCP) is a hybrid material created with a milling technique that fuses  $\beta$ -tricalcium phosphate and sodium lauryl sulphate or fumaric acid. This blending results in a functionalised TCP (fTCP). FTCP controls the delivery of calcium and phosphate ions to the teeth and works synergistically with fluoride to improve its performance.<sup>12</sup> Clinpro (3MESPE) is a recently introduced commercial fTCP paste available in the market.

Softening of the enamel surface is an early manifestation of the erosion process. Here, a scaffold of the hydroxyapatite crystal remains. At this stage agents like calcium, phosphorus and fluorides can bring about the remineralisation process. However, when the surface is completely lost, remineralising agents are not effective. In the initial stages of erosion, there is a reduction in the surface hardness of the enamel. Subsequent to remineralisation procedures, the hardness increases. Assessing surface hardness of enamel is therefore a useful tool to verify the efficacy of various remineralising agents.

Hence, the aim of the present study was to assess the enamel microhardness of teeth demineralised following exposure to an acidic beverages like carbonated drink - coca cola and evaluating the remineralising potential of recently available remineralising pastes, namely CPP-ACP (Tooth Mousse), Amine fluoride (Amflor) and NaF with functionalised tricalcium phosphate (Clinpro).

Material	Manufacturer	Composition	pН				
Colo Soft Drink	Com Colo	Carbonated water, 10 percent sugar,					
Cold Solt Dillik	COLA-COIA	Flavours					
		0.65 g/L potassium chloride British Pharmacopoeia (BP), 0.058 g/L					
		magnesium chloride BP, 0.165 g/L					
Artificial Saliva	Cash Pharmacy	calcium chloride BP, 0.804 g/L dipotassium hydrogen phosphate U.S.					
	(Bangalore)	pharmacopeia, 0.365 g/L potassium					
		dihydrogen phosphate, 2 g/L sodium benzoate, 7.8 g/L sodium carboxymethy					
		cellulose BP, deionized water to make 1 L.					
		Pure water, glycerol, casein phosphopeptide-amorphous calcium					
Casein		phosphate, d-sorbitol, silicon dioxide,					
Phosphopeptide-		sodium carboxymethyl cellulose, propylene glycol, titanium dioxide, xylitol,					
Amorphous Calcium	GC, Tokyo	phosphoric acid, guar gum, zinc oxide, sodium saccharin, ethyl-p-					
Phosphate (Tooth		hydroxybenzoate, magnesium oxide,					
Mousse		butyl-p-hydroxybenzoate, propyl-p-					
		hydroxybenzoate					
	Group	Purified water, sorbitol, propylene glycol, silica, amine fluoride (1000 ppm					
Amflor	Pharmaceuticals,	als, fluoride), cocamidopropyl betaine, titanium dioxide, peppermint oil,					
	Bangalore	hydroxyethyl cellulose, sodium saccharin.					
Clinpro		Water, Sorbitol, Hydrated Silica, Glycerin, Polyethylene-Polypropylene Glycol,					
	2M ECDE	Flavour, Sodium Lauryl Sulfate, titanium dioxide, carboxymethyl cellulose,					
	OM FOLF	sodium saccharin, sodium fluoride (950 ppm F <sup>-</sup> , 0.21% w/w, 0.12% w/v					
		fluoride ion), tri-calcium phosphate.					
Table 1. Materials Used, its Composition and pH							

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#### MATERIALS AND METHODS Sample Preparation

Thirty intact and noncarious human maxillary central and lateral incisors were collected and disinfected according to Occupational Safety and Health Administration (OSHA) recommendations. The teeth were stored in physiological saline and decoronated at cemento-enamel junction using a high speed diamond disc. Samples were divided into 3 main groups (n = 10 samples) with each group having two subgroups (n = 5 samples). They were mounted in self cure acrylic resin blocks with the labial surface levelled on top and lying flat and parallel to the horizontal plane and polished. A Vickers microhardness indenter (ZWICK/ROELL Indentec, India) was used to evaluate the baseline microhardness under 100 gm load applied for 15 seconds at 5 different points each 120 µm apart on the left-hand side of the middle one-third of the labial surface and the mean was measured. All the samples were stored in physiologic saline.

### **Erosion Process**

The pH of the cola soft drink (Coca-Cola, India) and artificial saliva (Table 1) was measured with a pH meter (ELCO, LI120, Bangalore). Each sample was immersed in 32.5 mL of the cola soft drink for five seconds and then in 32.5 mL of artificial saliva for another five seconds. Ten cycles of the immersion process were conducted at room temperature. This protocol was repeated two times at six-hour intervals. After the demineralisation process was completed, the samples were washed with deionized water and blotted dry. The enamel surface hardness was measured with the Vickers indenter at five different points and the mean was measured.

#### **Remineralisation Process**

A 0.5 mm layer of CPP-ACP (GC Tooth Mousse) was applied on the enamel surfaces of the samples in Group I for 3 min and then the samples were stored in artificial saliva (Group

# **Original Research Article**

IA) and deionized water (Group 1B). For Group II, 0.5 mm layer of amine fluoride (Amflor) was applied for 3 min and samples were stored in artificial saliva (Group IIA) and deionized water (Group IIB). For Group III, 0.5 mm layer of functionalised Tricalicum Phosphate (Clinpro) was applied 3 min and samples were stored in artificial saliva (Group IIIA) and deionized water (Group IIIB). All the samples were stored at respective solutions for six hours and at 37° C. After the remineralisation process was completed, the samples were washed with deionized water and blotted dry, followed by measurement of enamel surface hardness using Vickers indenter at five different points and the mean was calculated.

## **Statistical Analysis**

Statistical analysis was done using one-way repeated measures analysis of variance (ANOVA) to compare the Vickers Hardness Numbers (VHNs) at baseline, after erosion (demineralisation) and after remineralisation. The homogeneity was tested between groups at baseline and after erosion by using one-way ANOVA at a 95 percent confidence level. The differences between groups were tested after remineralisation by using Bonferroni method.

### RESULTS

The mean enamel microhardness at baseline after erosion and after remineralisation is shown in Table II and Graph 1. After remineralisation, the mean microhardness increased by 29.03% in Group IA (CPP-ACP in artificial saliva), 23.88% in Group IB (CPP-ACP in deionized water); 30.78% in Group IIA (Amflor in artificial saliva), 19.43% in Group IIB (Amflor in deionized water) and 30.04% in Group IIIB (Amflor in artificial saliva), 25.98% in Group IIIB (Clinpro in deionized water). There was no statistical significant difference among the groups when the P value is 0.05. Statistical difference was seen among the samples of Group IIA and Group IIB when the P value is 0.043.

Enamel Treatment	Group I A	Group I B	Group II A	Group II B	Group III A	Group III B	P value		
At Baseline	451.20±42.76	434.70±21.94	500.60±39.10	486.20±36.85	423.00±63.55	474.80±42.32	0.057		
After Demineralisation (Erosion)	361.60±25.74	327.40±46.03	376.20±18.13	326.60±59.68	364.80±22.44	341.80±45.07	0.250		
After Remineralisation	466.60±9.03	405.60±47.97	492.00±4.47	389.60±64.06	474.40±48.38	430.60±75.32	0.043		
Table 2. Mean Enamel Microhardness Values at Baseline, after Demineralisation and Remineralisation									



Graph 1. Column Graph showing the Comparison of Mean Microhardness of All the Groups in Different Storage Media

### DISCUSSION

In recent times, the prevalence of dental erosion is steadily increasing.<sup>13</sup> Management of erosion is thus becoming important to the long-term health of the human dentition. One of the main causes for enamel demineralisation is the drop in pH below the critical point for hydroxyapatite dissolution. The equilibrium between enamel demineralisation and remineralisation maintains an intact enamel surface.<sup>14</sup> Erosion is considered as a mineral loss. Mineral gain or loss in enamel as a result of demineralisation and remineralisation can be measured as a change in hardness.<sup>15</sup>

Reduced hardness of enamel and loss of mineral can be measured by various methods, which include direct and indirect techniques. Direct techniques are Longitudinal Microradiography (LMR), Transverse Microradiography (TMR) and Wavelength independent x-ray Microradiography (WMR). Indirect techniques include Polarised Light microscopy (PL), microhardness measurement methods and iodide permeability. Indirect methods are nevertheless quantitative and can measure changes in the real physical parameters. In case of polarised light, they can detect the general porosity of the enamel substrate. The use of surface microhardness tests can measure the change in surface structural strength.<sup>16</sup>

Microhardness test was selected in this study as it is simple, economical and also an effective method to evaluate and compare the demineralisation and remineralisation changes.<sup>17</sup> Vickers hardness testing was employed because the square shape of indent obtained in Vickers hardness testing is easy and more accurate to measure. Even the minute changes in the square shape indent obtained after the test can be easily detected.<sup>18</sup>

The baseline enamel microhardness values were ranged from 451.20 to 478.80 VHN. These values are higher than those reported by previous studies.<sup>15</sup> Cuy et al found that enamel hardness varies depending on the degree of mineralisation of enamel, local variations from enamel rods and tufts and increased porosity near the dentino-enamel junction.<sup>15</sup> The present study was done on polished enamel surface, which was similar to the studies done by Wongkhantee et al and Sukasame et al.

In the present study, artificial saliva was used as the storage medium after the erosion process in order to simulate the oral environment.<sup>15</sup> In many studies artificial saliva was employed as storage medium, because it can contribute to a slight increase in the microhardness after demineralisation.<sup>18</sup> Deionized water was used as the control group. We employed cyclic specimen immersion to simulate the washing effect of saliva in a person drinking a 325 mL cane of soft drink. The erosion process was performed three times at six-hour intervals to represent three meal times.<sup>15</sup> The mean reduction in enamel microhardness after erosion by carbonated drink (cola) was found to be 35.7 VHN. This was similar to the values reported by Sukasame et al, who also employed polished enamel surface in their study.<sup>15</sup>

The oral cavity is a battle field of activities of demineralisation and remineralisation. Tooth erosion shifts the equilibrium towards demineralisation. It is best managed if the demineralisation can be reversed in the initial stages itself by use of suitable remineralising agents. In recent times, a variety of remineralising agents are available like fluorides (inorganic and organic), casein phosphopeptides, xylitol, hydroxyapatite, etc.<sup>9</sup>

So the present study was conducted to compare enamel remineralisation by three different remineralising agents like CPP-ACP (Tooth Mousse), Amflor (Organic fluoride) and Clinpro (Inorganic fluoride + fTCP). The surface enamel microhardness increased by 29.03% and 23.88% in Group IA (CPP-ACP artificial saliva) and Group IB (CPP-ACP deionized water) respectively. CPP-ACP technology is based on ACP stabilised by Casein Phosphopeptides (CPP). Many studies have well documented the role of CPP-ACP in remineralisation of initial carious lesions. The main advantage of CPP-ACP is that it binds to tooth surfaces in the mouth rather than concentrating in the saliva. From here it modulates the bioavailability of calcium phosphate levels.<sup>9,19</sup> The bound CPP-ACP releases calcium ions, phosphate ions and hydroxide ions to diffuse into enamel subsurface lesions. Supersaturation of these ions within the enamel lesion leads to remineralisation of the enamel.<sup>20,21</sup> Recent studies have indicated that the anticariogenic properties of CPP-ACP may also be responsible for prevention of enamel erosion. CPP-ACP is an efficient remineralising agent, as it can consume the acid generated during enamel erosion by generation of increased levels of calcium and phosphate ions including CaHPO<sub>4</sub>, thus maintaining the high concentration gradients into the lesion.<sup>20,21</sup> The results of our study concurred with the results of previous studies, which have also reported increased remineralising potential of CPP-ACP on eroded enamel.<sup>15,22</sup>

Topical fluorides are popular agents for caries prevention and remineralisation of early lesions giving an acid resistant surface to the reformed crystals. Until recently inorganic fluorides like sodium fluoride, stannous fluoride, acidulated phosphate fluoride, etc. have been popular as remineralising agents. Presently, it has been demonstrated that organic fluorides like amine fluoride are superior to inorganic fluorides in this regard. There are not many studies on the effect of amine fluoride on enamel microhardness subsequent to demineralisation. A study by Arnold WH et al demonstrated superiority of amine fluoride (AmF) over sodium fluoride (NaF) due to slow release of fluorine and a more constant salivary fluorine level.<sup>14</sup> In our study, Group IIA (AmF in artificial saliva) demonstrated 30.78% increase in enamel microhardness. This may be because long-chain amines have been reported to potentiate fluoride uptake as well as retard acid dissolution of enamel.

Recently, in order to improve the remineralising efficacy of fluoride containing tooth pastes, attempts have been made to combine fluorine with other remineralising agents. This is because researchers have reported that non-fluoride mineralising agents like calcium and phosphate are capable of working synergistically with fluoride to elevate the efficacy of the combination.<sup>12,23</sup>

A novel fluoride dentifrice, Clinpro 5000, was introduced by 3M ESPE. This is a 1.1% NaF containing an innovative functionalised Tricalcium Phosphate (fTCP) ingredient that has been shown to boost remineralisation performance relative to fluoride only systems.<sup>12,24</sup> During the manufacturing process, a protective barrier is created around calcium allowing coexist with fluoride ions. As it comes in contact with saliva brushing, the barrier breaks down and makes the calcium, phosphate and fluoride readily available to the tooth.

A study by Karlinsey concluded that the synergistic combination of fluoride plus fTCP may provide superior surface and subsurface remineralisation of enamel compared to only fluoride toothpastes.<sup>12</sup> This is similar to the findings of our study where Group IIIA (Clinpro in artificial saliva) showed 30.78% and Group IIIB (Clinpro in deionised water) showed 25.98% respectively.

The results indicate that all the three remineralising agents tested are effective in reversing the effects of tooth erosion. Their remineralising potential is comparable. They demonstrated superior results in artificial saliva as compared to deionized water. Our observations should be strengthened with support from additional in vitro studies with larger sample size as well as long-term large scale clinical trials.

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## CONCLUSION

It can be concluded that microhardness of surface enamel is decreased by coca cola and all the remineralising agents tested demonstrate a significant increase in enamel microhardness almost approaching baseline values. Artificial saliva is a better storage medium for enhancing remineralisation. Amine fluoride demonstrated superior remineralising potential than CPP-ACP and sodium fluoride with fTCP, although this was not statistically significant.

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