

Comparative evaluation of two remineralizing agents in limiting dental erosion

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Abstract

Introduction: Enamel erosions are becoming increasingly important for long term dental health and have been associated with acidic attacks caused by consumption of beverages. Dental health care community has sought effective remineralizing agents to combat deleterious effects of carbonated drinks with acidic content.

Methods: In-vitro study of remineralization potential of CPP-ACPF and β -TCP was evaluated on human enamel sections with one control group (Gp I) and two experimental groups (Gp II and III, n=15 in all three groups). Specimens of groups II and III were treated with CPP-ACPF and β -TCP respectively for protective remineralization over 28 days. Thereafter specimens of all three groups were subjected to a simulated acid attack by a cola-based beverage over a period of 4 days. Energy Dispersive X-ray Analysis (EDAX) was used in pre and post experimental stages to evaluate elemental mineral content of enamel. One way ANOVA was used to evaluate results.

Results: Both agents resulted in remineralization and protection against acid demineralization. β -TCP was found to provide better remineralization and protection from acid attack than CPP-ACPF. The difference in both findings was statistically significant ($p < 0.05$).

Conclusions: The two agents in this in-vitro study were effective in their remineralization potential with β -TCP emerging a better agent. EDAX was found to be an effective analytical tool for such studies.

Keywords: Remineralizing agent, Remineralization, Dental erosion, Acidic erosion, Demineralization

Introduction

Human dental enamel has a crystalline porous structure that allows access of free ions into its deeper layers. Minerals forming enamel are constantly lost and regained in normal oral environment.⁽¹⁾ If this balance is adversely affected, demineralization occurs leading to deterioration of the enamel structure.⁽²⁾

In a homeostatic neutral environment, the hydroxyapatite crystals of enamel are in a dynamic equilibrium with free calcium and phosphate ions present in saliva. Demineralization is caused by acids that have intrinsic or extrinsic sources. Some acids are produced by bacteria colonizing oral cavity by metabolizing carbohydrates, and some are introduced into the mouth as a part of food or drinks. The demineralization is reversible if pH is neutralized and there is sufficient bioavailability of calcium and phosphate ions in the immediate environment.⁽³⁾

Dental erosions are caused by acid dissolution of tooth surface without bacterial involvement. The process is also termed demineralization and may be caused by extrinsic or intrinsic agents. Extrinsic agents causing dental erosions are of greater importance to dental healthcare community as these account for this morbidity for the most part. Primary extrinsic causal agents have been identified as dietary acids from foodstuff, beverages and snacks, the consumption of which is increasing at an ever faster pace. It has been reported that dietary acids are the most important extrinsic factor.⁽³⁻⁵⁾ In essence, these are a present day health hazard associated with modern lifestyle.

There has been a twofold increment in consumption of soft drinks over the past few decades, especially among adolescents and young adults.⁽⁴⁻⁶⁾ Carbonated soft drinks contain carbonic acid and citric acid which are frequently added to improve taste of these beverages. Citrate anions in these solutions chelate calcium ions, decreasing the amount of free ionic calcium available in saliva and at enamel surface, thereby enhancing demineralization and limiting the potential for remineralization.⁽⁶⁾

As human longevity improves, dental erosions are becoming increasingly important to long-term dental health. It is therefore reasonable for dental health industry to search for effective agents for prevention or repair of these erosions. Fluoride has long been used as an anti-erosive agent. However, some studies have pointed out limitations of fluoride efficiency in this regard.⁽⁷⁾ It has also been stated that inclusion of minerals such as calcium and phosphate in fluoride preparations may enhance their anti-erosion benefits.^(7,8)

Fluoride and mineral synergistic products having anti-erosive properties such as fluoride-enriched casein phosphor-peptide-amorphous calcium phosphate (CPP-ACPF) and β -tricalcium phosphate have been introduced as improved agents for dental enamel remineralization.⁽⁷⁾

These agents effect remineralization of the teeth by various mechanisms. β -TCP causes remineralization of eroded enamel by providing calcium, phosphate and fluoride which react with rarefied enamel to provide a seed for enhanced mineral growth.⁽⁷⁾ CPP-ACPF tends to stabilize calcium phosphate as metastable solution which

buffers free calcium and phosphate ion activities, thus helping maintain a state of supersaturation which inhibits demineralization and enhances remineralization of enamel.⁽⁹⁻¹⁰⁾

This in vitro study was aimed at evaluating effect of two remineralizing agents on the enamel surface in resisting acidic attack of beverages.

Materials and Methods

This study was carried out on dental enamel sections (sample, n=45) obtained by sectioning 12 extracted molars having intact and untreated enamel surface morphology. These sections from buccal, lingual, mesial and distal surfaces of molars measured 5x5x2mm each. A small 3x3mm segment of modelling wax was placed at the centre of the external surface of all enamel sections [Fig. 1] and the remaining area was coated with acid resistant nail varnish. The wax block was removed to expose the experimental surface. The sample was divided in 3 groups (I, II and III; n=15 each). All sample specimens were marked group-wise and serially on the reverse surface for identification. Specimens remained stored in artificial saliva throughout the period of study, excluding experimental times during application of various agents.



Fig. 1: 3x3mm window on enamel section

Group I served as control and was subjected to EDAX (energy dispersive x-ray) analysis before conducting the experiment, to obtain quantitative elemental composition of enamel (Fig. 2, 3). No surface treatment was done for this group. In experimental groups, specimens of Group II were treated with CPP-ACPF and Group III specimens were treated with β -TCP.



Fig. 2: EDAX Unit

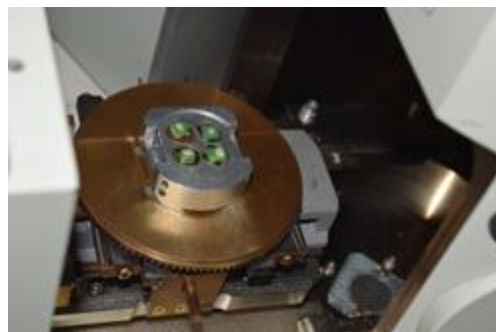


Fig. 3: Mounted Specimens for Scan

Groups II and III were treated with specified remineralizing agents for 4 minutes twice daily for 28 consecutive days, 12 hours apart; were rinsed after each application and then replaced in artificial saliva. Specimens were again subjected to EDAX analysis at the end of 28 days, to obtain quantitative elemental composition.

After pre-treatment of Group II and Group III with remineralizing agents for 28 days, all 45 specimens of groups I, II and III were immersed in 5 ml cola based beverage for 10 minutes for 4 consecutive days. After immersion in the beverage, all specimens were not rinsed and were directly stored in the artificial saliva. All 45 specimens were thereafter again subjected to EDAX analysis to obtain atomic weight percentage of calcium and phosphorous.

The data obtained on atomic weight percentage of calcium and phosphorous before and after remineralization and after demineralization was then subjected to statistical analysis.

This study was conducted at the Department of Conservative Dentistry and Endodontics, Bharati Vidyapeeth Deemed University Dental College and Hospital, Pune. Energy Dispersive X-ray Analysis was carried out at Faculty of Technology and Engineering, Department of Metallurgical and Materials Engineering, The Maharaja Sayajirao University of Baroda.

Results

Atomic element levels of calcium (Ca) and phosphorous (P) obtained with EDAX were expressed in percentage (%) for all three groups and the complete data range was tabulated [Table I]. The mineral content of mineralized and demineralized specimens of all three groups along with their Mean and SD was also tabulated (Table 2).

Between-group differences in Calcium/Phosphorous (Ca/ P) ratios after remineralization were analyzed using one way ANOVA test of significance with Bonferroni correction (Tables 3). These between-group differences in Ca/ P ratios after demineralization were also analyzed using one way ANOVA with Bonferroni correction (Tables 4). In the above analyses, p-value less than or equal to 0.05 ($p \leq 0.05$) was taken to be statistically significant. All

analyses were performed using SPSS software version 17.

Table 1: EDAX Complete Results: Calcium and Phosphate levels after experiments

S. No.	Ca Untreated	P Untreated	Ca after Remin	P after Remin	Ca after Demin	P after Demin
Group I [Control]						
1	58.47	41.53			57.35	42.65
2	53.99	46.01			52.24	47.76
3	57.83	42.17			56.82	43.18
4	59.58	40.42			58.47	41.53
5	59.12	40.88			57.86	42.14
6	58.49	41.51			57.53	42.47
7	58.96	41.04			57.69	42.31
8	59.74	40.26			58.35	41.65
9	59.66	40.34			58.73	41.27
10	58.79	41.21			57.96	42.04
11	60.61	39.39			58.0	42.0
12	59.53	40.47			58.21	41.79
13	58.81	41.19			57.1	42.9
14	57.98	42.02			56.0	44.0
15	59.21	40.79			58.32	41.68
Group II [CPP- ACP]						
16	58.47	41.53	59.16	40.84	59.63	40.37
17	53.99	46.01	55.85	44.15	55.11	44.89
18	57.83	42.17	58.46	41.54	58.88	41.12
19	59.58	40.42	61.45	38.55	59.06	40.94
20	59.12	40.88	60.88	39.12	59.15	40.85
21	59.49	41.51	61.37	38.63	59.92	40.08
22	58.96	41.04	60.54	39.46	59.88	40.12
23	59.74	40.26	60.02	39.98	59.83	40.17
24	59.66	40.34	60.12	39.88	60.00	40.00
25	58.79	41.21	59.00	41.00	59.37	40.63
26	60.61	39.39	61.22	38.78	61.02	38.98
27	59.53	40.47	60.59	39.41	60.14	39.86
28	58.81	41.19	59.55	40.45	58.43	41.57
29	57.98	42.02	60.26	39.74	59.78	40.22
30	59.21	40.79	60.65	39.35	60.21	39.79
Group III [β-TCP]						
31	58.47	41.53	61.53	38.47	61.27	38.73
32	53.99	46.01	58.51	41.49	57.33	42.67
33	57.83	42.17	60.44	39.56	59.86	40.14
34	59.58	40.42	60.48	39.52	60.19	39.81
35	59.12	40.88	61.37	38.63	60.93	39.07
36	58.49	41.51	61.22	38.78	60.59	39.41
37	58.96	41.04	60.43	39.57	60.17	39.83
38	59.74	40.26	62.02	37.98	61.81	38.19
39	59.66	40.34	61.11	38.89	59.93	40.07
40	58.79	41.21	60.29	39.71	60.06	39.94
41	60.61	39.39	62.21	37.79	61.88	38.12
42	59.53	40.47	61.66	38.34	61.03	38.97
43	58.81	41.19	62.72	37.28	61.49	38.51
44	57.98	42.02	61.64	38.36	61.01	38.99
45	59.21	40.79	62.68	37.32	62.17	37.83

Table 2: Mean, SD and Ratio of mineral content of remineralized and demineralized samples

		Untreated	Post demineralization
Group I Control	Ca (%), Mean \pm SD	58.72 \pm 1.49	57.38 \pm 1.59
	P (%), Mean \pm SD	41.28 \pm 1.49	42.62 \pm 1.59
	Ca/P ratio	1.16 \pm 0.14	1.02 \pm 0.07
		Post remineralization	Post demineralization
Group II CPP-ACPF	Ca (%), Mean \pm SD	59.94 \pm 1.44	59.36 \pm 1.33
	P (%), Mean \pm SD	40.06 \pm 1.44	40.64 \pm 1.33
	Ca/P ratio	1.38 \pm 0.25	1.19 \pm 0.20
		Post remineralization	Post demineralization
Group III β-TCP	Ca (%), Mean \pm SD	61.22 \pm 1.09	60.65 \pm 1.18
	P (%), Mean \pm SD	38.78 \pm 1.09	39.35 \pm 1.18
	Ca/P ratio	1.60 \pm 0.13	1.37 \pm 0.23

Table 3: Comparison of Ca/P ratio Post-Remineralization (All Groups)

	Control Group I (Untreated)	CPP-ACPF Group II	β -TCP Group III
Ca/P ratio (Mean \pm SD)	1.16 \pm 0.14	1.38 \pm 0.25	1.6 \pm 0.13
p value (One Way ANOVA) <0.001*			
Post hoc, Bonferroni test			
Control Group	-	0.029*	<0.001*
CPP-ACPF group	-	-	0.038*
β -TCP Group	-	-	-

* p < 0.05 is statistically significant

Table 4: Comparison of Ca/P ratio Post-Demineralization (All Groups)

	Control Group I	CPP-ACPF Group II	β -TCP Group III
Ca/P ratio (Mean \pm SD)	1.02 \pm 0.07	1.19 \pm 0.20	1.37 \pm 0.23
p value (One Way ANOVA) <0.001*			
Post hoc, Bonferroni test			
Control Group	-	0.048*	<0.001*
CPP-ACPF group	-	-	0.05*
β -TCP Group	-	-	-

* p < 0.05 is statistically significant

Discussion

Enamel is the hardest substance in human body. It remains in a dynamic balanced state of cycles of demineralization and remineralization. If the balance is disturbed and demineralization process predominates, it may eventually lead to development of carious lesions in enamel and dentine.⁽³⁾ In a normal physiologic environment, the hydroxyapatite crystals of enamel are in a dynamic equilibrium with calcium and phosphate ions. Dental erosion is a localized loss of tooth surface by a chemical process of acidic dissolution without the involvement of bacteria. Demineralization caused by acidic environment is reversible if there are sufficient bioavailable calcium and phosphate ions in the immediate vicinity.^(3,4,6)

Acids which cause dental erosion may be extrinsic or intrinsic. Extrinsic causes include intake of acidic substances, beverages, foods, medication and environmental exposure to acidic agents. Intrinsic causes of erosion include recurrent vomiting as part of the eating disorders such as anorexia or bulimia or due to regurgitation of gastric contents.^(3,5)

Dietary acids have been extensively studied aetiological agents and stated to be the most important extrinsic factor. The prevalence of erosion is believed to be on the rise, reflecting the availability and frequent consumption of acidic beverages, fruit juices, wines, sport drinks. Several studies have reported a strong association between dental erosion and acidic foodstuff and soft drinks.⁽²⁻⁵⁾

Consumption of packaged food and carbonated drinks is popular in younger age groups today.⁽⁶⁾ Lack of dietary awareness has become an important issue in modern society. Prevalence of dental erosion is increasing and soft drink consumption is recognized as a prime etiological factor. Many clinical studies have found soft drinks, especially carbonated cola drinks, to be associated with erosion; most likely due to their low pH. The erosive potential of soft drinks within first few minutes of exposure is solely a function of the pH of these drinks. In several studies the erosive potential was found to be manifold higher in cola drinks when compared with orange juices.⁽²⁻⁶⁾ In this study, cola based beverage was used to induce enamel demineralization as it is one of the most commonly consumed acidic beverage. Hermetically sealed cola containers were used because escape of dissolved gas from the drink may increase its pH and decrease its potential of dissolving hydroxyapatite. The specimens were stored in artificial saliva throughout the period of study to simulate oral conditions.

In this study, EDAX (Energy Dispersion X-ray Analysis) was used for elemental analysis at ultra-structural level. It is a micro-analytical technique that is used along with SEM wherein SEM does the structural analysis and EDAX carries out elemental analysis.⁽¹⁾ The principle is based on the energy emitted in the form of element-specific x-ray photons when electrons from external sources collide with the atoms in an element, thus generating characteristic x-rays of that element. When the sample is bombarded by the electron beam of the SEM, electrons are ejected from the atoms on the specimen's surface (secondary electrons). A resulting electron vacancy is filled by an electron from a higher shell, and an X-ray is emitted (characteristic X-rays) to balance the energy difference between the two electrons. The EDAX x-ray detector measures the number of emitted x-rays and their energy. The energy of the x-ray is characteristic of the element from which the x-ray was emitted. A spectrum of the energy vs relative counts of the detected x-rays is obtained and evaluated for qualitative and quantitative determinations of the elements present in the specimen using a computer-based program.⁽¹⁾

The present study evaluated and compared the effect of two remineralizing agents on the enamel surface in resisting acidic attack of beverages using EDAX. The enamel specimens for the study were prepared to have a small 3x3mm experimental surface so as to minimize area of exposure for EDAX and enhance accuracy.

Two remineralizing agents – CPP-ACPF and β -TCP were used in this study. The remineralizing paste was applied on the specimens for 4 minutes twice daily for 28 days. It has also been proved in previous studies that longer the duration of remineralizing agent in contact with the teeth, better was the remineralization.⁽¹¹⁻¹³⁾

The result of this study showed that both remineralizing agents were able to provide protective

effect against enamel erosion. Group III specimens (pre-treated with β -TCP) showed better protective effect than Group II specimens (pre-treated with CPP-ACPF). It was found that Ca/P ratio after remineralization and demineralization of enamel samples was statistically higher in Group III compared to Groups I and II. [(Ca/P ratio after remineralization for β -TCP is 1.6, CPP-ACPF is 1.38, Control is 1.16) (Ca/P ratio after demineralization for β -TCP is 1.37, CPP-ACPF is 1.19, Control is 1.02)]. The Ca/P ratio after remineralization and demineralization of the specimens in Group III showed statistically significant difference when compared to Group I and Group II.

β -TCP is a hybrid material formed by fusion of tricalcium phosphate and sodium lauryl sulphate. This blending results in weakly bonded calcium and a 'free' phosphate, designed to increase the efficiency of fluoride remineralization. β -TCP is also similar to hydroxyapatite structure and possesses unique calcium characteristics capable of reacting with fluoride and enamel. When phosphate ions float freely, the exposed calcium bonds are protected by preventing the calcium from prematurely interacting with fluoride. β -TCP is designed to coexist with fluoride in a mouth rinse or dentifrice over its shelf-life as it will not react before reaching the target tooth surface. When β -TCP comes into contact with the tooth surface and is moistened by saliva, the protective barrier breaks down, making the calcium, phosphate and fluoride ions available to the teeth. The fluoride and calcium then reacts with weakened enamel to provide seeding for enhanced mineral growth relative to fluoride alone. Studies have concluded that β -TCP provided superior surface and subsurface remineralization compared with a 5000 ppm fluoride and CPP-ACP combination.⁽¹⁴⁻¹⁶⁾

In our study, β -TCP showed better protective effect than CPP-ACPF. This result is similar to that showed in a study by Patil et al⁽¹⁶⁾ where TCP+fluoride-based products performed better than CPP-ACP-based products in remineralizing artificial enamel caries. The reason could possibly be that a higher concentration of calcium ion in TCP and addition of fluoride might have led to better remineralization capacity than CPP-ACPF.

Group II in this study was treated with CPP-ACPF. The Ca/ P ratio after remineralization and demineralization of specimens in this group was statistically more as compared to Control group but was less when compared to β -TCP group.

Casein phosphopeptide-amorphous calcium phosphate is a nanocomplex derived from bovine milk protein, casein. The CPP-ACP preparations have also been reported to significantly remineralize enamel subsurface lesions in vitro.⁽¹¹⁾ CPP binds to nanoclusters of ACP through multiple phosphoseryl residues, preventing their growth to the critical size required for nucleation and phase transformation. CPP facilitates high concentrations of calcium and phosphate ions by stabilizing calcium phosphate including CaHPO_4 ; which

can diffuse into the enamel subsurface lesion. CPP-ACP has been extensively incorporated into oral health products such as mouth rinses, chewing gums, and a sports drinks to reduce enamel erosion. It is well known that the localization of ACP at the tooth surface buffers the free calcium and phosphate ion activities, thus helping to maintain a state of local super saturation which inhibits demineralization and promotes remineralization of the enamel.

The process of remineralization involves diffusion of calcium and phosphate ions through the protein/water-filled pores of the carious enamel surface into the body of the enamel lesion. Once in the body of the enamel lesion, these calcium and phosphate species increase the activities of Ca^{2+} and PO_4^{3-} , thus increasing the degree of saturation with hydroxyapatite.^(17,18)

In the present study CPP-ACP fluoride was used. CPP-ACPF showed better protective effect than the specimens pretreated with Control group which is found to be similar to other studies^(16,19-21) In our study CPP – ACPF group was second to β -TCP as far as remineralization potential is concerned.

Thus from the results obtained from this study it can be suggested that the remineralizing agents can impede acidic attack of beverages on enamel. However this is an in vitro study and remineralization in vitro may be quite different from what happens with dynamic complex biologic system which prevails in oral cavity in vivo.^(22,23) Thus direct extrapolations to clinical conditions must be exercised with caution because of obvious limitations of in-vitro studies.

Conclusions

Within the limitations of this in-vitro study following conclusions were made:

1. The two remineralizing agents tested were found to be effective in inhibiting the demineralization caused by cola-based beverage.
2. β -TCP was found to be more effective than CPP-ACPF in remineralization.
3. Energy dispersive X-ray analysis was found to be an efficient way to quantitatively assess the changes in mineral content.

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