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RESEARCH ARTICLE

PREVENTIVE ROLE OF β-TCP IN DENTAL EROSION DUE TO BEVERAGE INTAKE

Anupam S Sharma., Chandni Patel and Jyoti Mandlik

Department of Conservative Dentistry & Endodontics, Bharati Vidyapeeth Deemed University Dental College & Hospital, Pune - 411043, India

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ABSTRACT

Aim : To evaluate efficacy of β-TCP in prevention of tooth enamel demineralisation caused by intake of carbonated beverage.
Materials and Methods: In-vitro remineralisation potential of β-TCP was studied on a sample of human dental enamel sections against a control group (n=15 each). Specimens of experimental group were treated with β-TCP for protective remineralisation over 28 days. Thereafter specimens of both control and experimental groups were subjected to a simulated acid attack by a cola-based beverage over a period of 4 days. Energy Dispersive Xray 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: β-TCPwas found to effect remineralisation as well as protection against acid demineralisation. The difference in both findings was statistically significant ($p=<0.05$).
Conclusion: β-TCP was found to be effective in its remineralisation potential and protection against acidic attacks of carbonated beverage. EDAX was found to be an effective analytical tool for such studies.

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, demineralisation 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. Demineralisation is caused by acids that have intrinsic or extrinsic sources. Some acids are produced by bacteria colonizing oral cavity by metabolising carbohydrates, and some are introduced into the mouth as a part of food or drinks. The demineralisation 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 demineralisation and may be caused by extrinsic or instrinsic 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 demineralisation and limiting the potential for remineralisation.[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 β-tricalcium phosphate have been introduced as improved agents for dental enamel remineralisation.[7] β-TCP causes remineralisation of eroded enamel by providing calcium, phosphate and fluoride which react with rarefied enamel to provide a seed for enhanced mineral growth.[7-10]

This in vitro study was aimed at evaluating effect of β-TCP on dental enamel surface in resisting acidic attack of beverages.

MATERIALS AND METHODS

This study was carried out on dental enamel sections (sample, n=30) obtained by sectioning 10 extracted molars having intact and untreated enamel surface morphology. Each section from buccal, lingual, mesial and distal surfaces of molars measured 5x5x2mm. 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 samples were divided in 2 groups [I (control) and II (experimental), 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 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.

Fig. 2 EDAX Unit

Fig. 3 Mounted Specimens for Scan

In experimental group II, specimens were treated with β-TCP 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 pretreatment of Group II with remineralizing agent for 28 days, all 30 specimens of groups I and II 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 30 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 remineralisation and after demineralisation 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 (%) and ratios for both groups [Tables 1 and 2]. Between-group differences in the Calcium/Phosphorous (Ca/P) content and Ca/P ratios after remineralisation and demineralisation were analyzed using one way ANOVA test of significance with Bonferroni correction [Tables 3 and 4]. In the above test, 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

Sr 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 [β-TCP]						
16	58.47	41.53	61.53	38.47	61.27	38.73
17	53.99	46.01	58.51	41.49	57.33	42.67
18	57.83	42.17	60.44	39.56	59.86	40.14
19	59.58	40.42	60.48	39.52	60.19	39.81
20	59.12	40.88	61.37	38.63	60.93	39.07
21	58.49	41.51	61.22	38.78	60.59	39.41
22	58.96	41.04	60.43	39.57	60.17	39.83
23	59.74	40.26	62.02	37.98	61.81	38.19
24	59.66	40.34	61.11	38.89	59.93	40.07
25	58.79	41.21	60.29	39.71	60.06	39.94
26	60.61	39.39	62.21	37.79	61.88	38.12
27	59.53	40.47	61.66	38.34	61.03	38.97
28	58.81	41.19	62.72	37.28	61.49	38.51
29	57.98	42.02	61.64	38.36	61.01	38.99
30	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 demineralisation
Group I Control	Ca (%), Mean ± SD	58.72 ± 1.49	57.38 ± 1.59
	P (%), Mean ± SD	41.28 ± 1.49	42.62 ± 1.59
	Ca/P ratio	1.16 ± 0.14	1.02 ± 0.07
		Post remineralisation	**Post demineralisation**
Group II β-TCP	Ca (%), Mean ± SD	61.22 ± 1.09	60.65 ± 1.18
	P (%), Mean ± SD	38.78 ± 1.09	39.35 ± 1.18
	Ca/P ratio	1.60 ± 0.13	1.37 ± 0.23

Table 3 Comparison of Ca/P ratio Post-Remineralisation (Groups I and II)

	Control Group I (Untreated)	β-TCP Group II
Ca/P ratio (Mean ± SD)	1.16 ± 0.14	1.6 ± 0.13
p value (One Way **ANOVA**)	<0.001*	
Post hoc, **Bonferroni test**		
Control Group	-	<0.001*
β -TCP Group	-	-

* $p < 0.05$ is statistically significant

Table 4 Comparison of Ca/P ratio Post-Demineralisation (Groups I and II)

	Control Group I (Untreated)	β-TCP Group II
Ca/P ratio (Mean ± SD)	1.02 ± 0.07	1.37 ± 0.23
p value (One Way **ANOVA**)	<0.001*	
Post hoc, **Bonferroni test**		
Control Group	-	<0.001*
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 demineralisation and remineralisation. If the balance is disturbed and demineralisation 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. Demineralisation 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 demineralisation 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 Xray Analysis) was used for elemental analysis at ultrastructural level. It is a microanalytical 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 the effect of β-TCP as a remineralizing agent 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. 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 remineralisation.[11-13]

The result of this study showed that β-TCP was able to provide protective effect against enamel erosion. Group II specimens (pretreated with β-TCP) demonstrated having protective effect compared to untreated control Group I specimens. It was found that Ca/P ratio after remineralisation and demineralisation of enamel samples was statistically higher in Group II compared to Groups I. Ca/P ratio after remineralisation for β-TCP was 1.6 and for control was 1.16. The same ratio after demineralisation for β-TCP was 1.37 and for control was 1.02.

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 remineralisation. 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 mouthrinse 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 remineralisation compared with a 5000 ppm fluoride and other combinations.[14-16]

In our study, β-TCP showed significant protective effect. This result is similar to that showed in a study by Patil *et al*[16] where TCP + fluoride-based products performed better than other calcium-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 remineralisation capacity.

The process of remineralisation 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]

In the present study β-TCP showed both remineralisation effect as well as protective effect against demineralisation consequent to acidic attack when compared to untreated control group, which is found to be similar to other studies.[18-21]

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 remineralisation in vitro may be quiet 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. β-TCP as remineralizing agent was found to be effective in inhibiting the demineralisation caused by cola-based beverage.
2. β-TCP application was also found to effect remineralisation of enamel surface.
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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