



A comparative evaluation of the effect of alcoholic and non alcoholic beverages on tooth enamel surface pretreated with β -tricalcium phosphate, bioactive glass and amine fluoride: an *in vitro* study

1) Department of Conservative Dentistry and Endodontics, College of Dental Science and Hospital, Rau, Indore, India

2) KLE VK Institute of Dental Sciences, Nehru Nagar, Belagavi, Karnataka, India

Anjal Shah¹, Hemalatha Hiremath², Kirti Ojha¹, Sheetal Khandelwal¹, Sonam Patidar¹, Shivangi Trivedi¹

Abstract

Background and aims. The aim of this *in vitro* study is to quantitatively evaluate the effect of different alcoholic and non alcoholic beverages on the tooth enamel surface topography pretreated with various remineralizing agents using Atomic Force Microscopy.

Methods. 120 tooth specimens were prepared from 60 freshly extracted intact human premolars by sectioning from mesial to distal surfaces using low speed diamond discs and were randomly assigned to study groups and control group. Specimens of Group I, Group II and Group III were pre-treated with β -Tri calcium phosphate, bioactive glass and amine fluoride respectively for 4 minutes for 28 days, followed by storage in artificial saliva. All the specimens were evaluated for surface roughness using Atomic Force Microscopy. The specimens were then placed in alcoholic and non-alcoholic beverages for 10 minutes for 4 days and were again analyzed by Atomic Force Microscopy.

Descriptive statistics was performed by using the proportional or frequency distribution of the parameters. The respondents were then grouped according to the branch of specialty if any and the data was evaluated by the one-way ANOVA with post-hoc, with p value <0.005.

Results. In the present study, among the remineralizing agents tested, bioactive glass was found to be more effective than β -Tri Calcium Phosphate and Amine Fluoride. Among the demineralizing agents used in this study, the demineralization potential of Coca Cola was found to be highest, followed by wine and green tea pretreated with β -tricalcium phosphate, bioactive glass and amine fluoride.

Conclusions. The present study concluded that all the remineralizing agents tested were found to be effective in inhibiting the demineralization caused by various alcoholic and non alcoholic beverages. Among the remineralizing agents tested, bioactive glass was found to be more effective than β -tri calcium phosphate and amine fluoride.

Keywords: beta-tricalcium phosphate, colabased beverage, CPP-ACPF, demineralization, remineralization

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Address for correspondence:

Anjal Shah

dranjajain@gmail.com

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Introduction

Dental erosion is defined as an irreversible loss of dental hard tissue caused by a chemical process. The tooth enamel is a microcrystalline porous structure that allows the entry of ions extensively into its deeper layers. Minerals forming enamel are lost and regained continuously in normal oral environment [1].

Tooth undergoes continuous demineralization and remineralization in the oral environment. If this balance is disrupted, the demineralization process leads to deterioration of the hard tissue structure like enamel and dentin [2].

The process of caries initiates with demineralization of the subsurface tooth enamel. Loss of calcium and phosphate ions from enamel results in formation of a lesion underneath. At this early stage, the carious lesion is reversible via a remineralization process involving the diffusion of calcium and phosphate ions below the surface of the lesion to restore the lost tooth structure [3].

Remineralization is a natural repair process which depends on calcium, phosphate ions and fluoride to rebuild a new surface on existing crystal remnants after demineralization. The amount of inorganic ions and its composition affects the degree of saturation of the fluid in saliva [4].

The mineral in the dentin and enamel is not pure hydroxyapatite, but rather a mixture of compounds including a number of carbonated apatites. The chemical inhomogeneity of enamel makes the process of enamel remineralization rather complex. When the pH is neutral and there are sufficient Ca^{2+} and PO_4^- ions in the oral environment, the demineralization process can be reversed and remineralization occurs [1].

Demineralization in the oral cavity can be attributed mainly to dental caries. In addition to it, various intrinsic and extrinsic erosive factors are also responsible. Extrinsic agents include acidic carbonated beverages, foods like chewing gums, acidic candies, snacks, and may also depend upon environment, lifestyle and medications. Intrinsic erosion is associated with gastric acid which may be present intra-orally following vomiting, regurgitation and gastro-esophageal reflux [5-9].

Recently, fluoride containing newer formulations such as β -tricalcium phosphate (Clinpro™ Tooth Crème), bioactive glass (BAG) (SHY- NMGroup Pharmaceuticals; India) and amine fluoride (Enafix tooth paste, Group Pharmaceuticals; India) which is a new generation organic fluoride have been introduced as newer products for remineralization.

Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) nanocomplexes which have been demonstrated to have anticariogenic as well as anti-erosive properties.

Therefore, the management of demineralized lesions include the use of oral products containing CPP-ACP and

fluoride. CPP-ACP binds to biofilms, plaque, bacteria, hydroxyapatite and soft tissue localizing bio-available calcium, phosphate and fluoride [10].

Functionalized tricalcium phosphate (fTCP) mineral comprises of β -TCP and sodium lauryl sulfate (SLS) with fluoride level of 950 ppm. The bioavailable calcium is protected by the fTCP technology with a fluoride-repelling surfactant [11].

Novamin/BAG is a unique material. It has numerous novel features; the most important feature is its ability to act as a biomimetic mineralizer, matching the body's own mineralizing traits. Sodium ions from the BAG particles are rapidly exchanged with hydrogen cations (in the form of H_3O^+) present in saliva and this brings about the release of calcium and phosphate (PO_4) ions from bioactive glass [12].

Several studies have been reported on the use of remineralizing agents in the management of demineralization arising from dental caries. However, to the best of our knowledge few studies reported on role of remineralizing agents in preventing the loss of minerals from the tooth structure and even fewer on the effect of alcoholic and non-alcoholic beverages on enamel surface previously treated with remineralizing agents. Hence, the present in vitro study was carried out to evaluate and compare the effect of different alcoholic and non alcoholic beverages on the tooth enamel surface topography pretreated with various remineralizing agents [13,14].

Methods

Sixty intact human premolars which were extracted for orthodontic/periodontal reasons were freshly collected and disinfected in 10% thymol solution for 24 hours as per CDC (Centre for Disease Control and Prevention) guidelines and stored until the samples were used.

Buccal surfaces of premolar teeth were marked (line extending in mesio-distal direction) with pencil using a metallic scale and then sectioned at CEJ with the help of diamond disc (Kerr Dental, Medium size) using a low – speed handpiece from mesial to distal surface. The crown samples were included in the study. The samples were divided into 4 groups (n=30) A, B, C and D based on the remineralizing agents used. The remineralizing agent was applied using paint brush, twice daily for 4 mins for 28 days. The samples were stored in artificial saliva to simulate the oral conditions.

Samples of group A were remineralized with β -Tri calcium Phosphate (Clinpro Tooth Crème; 3M ESPE), samples of group B were remineralized with bioactive glass (SHY-NM; Group Pharmaceuticals), samples of group C were remineralized with amine fluoride (Enafix Toothpaste; Group Pharmaceuticals) and Group D was control. The samples were then analyzed at 3 points (cervical, middle and incisal) using Atomic Force

Microscopy to measure the surface roughness.

Each group was further divided into 3 sub-groups of 10 teeth each based on the demineralizing agents used (wine, coca cola and green tea). The samples were again analyzed in the same area as previous at the 3 points (cervical, middle and incisal) using Atomic Force Microscopy to measure the surface roughness. Mean value was statistically analyzed by one-way analysis of variance (ANOVA) at 5% level of significance.

One way ANOVA was applied to compare the mean difference between mean values of any parameters between three and more groups, followed by POST Hoc Tukey test for individual group comparison. The P Value < 0.05 will be considered as level of significance.

Microsoft Excel and R Studio (Open source analytical tool V 1.2.335) was used to collect the data, perform the basic calculation, statistical analysis and

presentation of results.

Results

Statistical analysis was done using one way ANOVA test to compare the surface roughness of experimental groups after remineralization (Table I) followed by Post hoc Tukey test for pairwise comparison of mean value of experimental groups (Table II). Comparison of root mean square surface roughness was done among different beverages after demineralization process (Table III) followed by pairwise comparison of mean value of subgroups (Table IV).

Upon comparison of average values among all the groups, bioactive glass showed more remineralization potential than β -TCP and amine fluoride. The demineralization potential values were the highest in coca cola followed by wine and green tea.

Table I. Comparison of mean values among different pastes after remineralization process (in nm).

Remineralization Paste	N	Mean	Std. Deviation	F Test	P Value	Result
β -TCP	30	150.979	3.042	3383.530	0.000	Sig
Bioactive Glass	30	133.308	2.114			
Amine Fluoride	30	161.574	1.766			
Control	30	188.854	1.527			
Total	120	158.679	20.336			

Table II. Pairwise comparison of mean value of experimental groups (in nm).

Remineralizing pastes		Mean Difference	Std. Error	P Value	Result
β -TCP	BioActive Glass	17.672	0.565	0.000	Sig
	Amine Fluoride	-10.595	0.565	0.000	Sig
BioActive Glass	Amine Fluoride	-28.267	0.565	0.000	Sig
	β -TCP	37.874	0.565	0.000	Sig
Control	BioActive Glass	55.546	0.565	0.000	Sig
	Amine Fluoride	27.279	0.565	0.000	Sig

Table III. Comparison of root mean square surface roughness among different beverages in group A, B, C samples after demineralization process (in nm).

Beverages	β -TCP			Bioactive glass			Amine Fluoride		
	N	Mean	P Value	N	Mean	P Value	N	Mean	P Value
Wine	10	195.437	0.000	10	162.589	0.000	10	213.699	0.000
Coca Cola	10	203.415		10	181.194		10	243.357	
Green tea	10	48.693		10	46.754		10	57.500	
Total	30	149.182		30	130.179		30	171.519	

Table IV. Comparison of mean value among subgroups (in nm).

Beverages		β -TCP		Bioactive glass		Amine fluoride	
		Mean Difference	P Value	Mean Difference	P Value	Mean Difference	P Value
Wine	Coca cola	-7.979	0.485	-18.604	0.014	-29.658	0.121
	Green tea	146.744	0.000	115.836	0.000	156.199	0.000
Coca Cola	Green tea	154.723	0.000	134.440	0.000	185.857	0.000

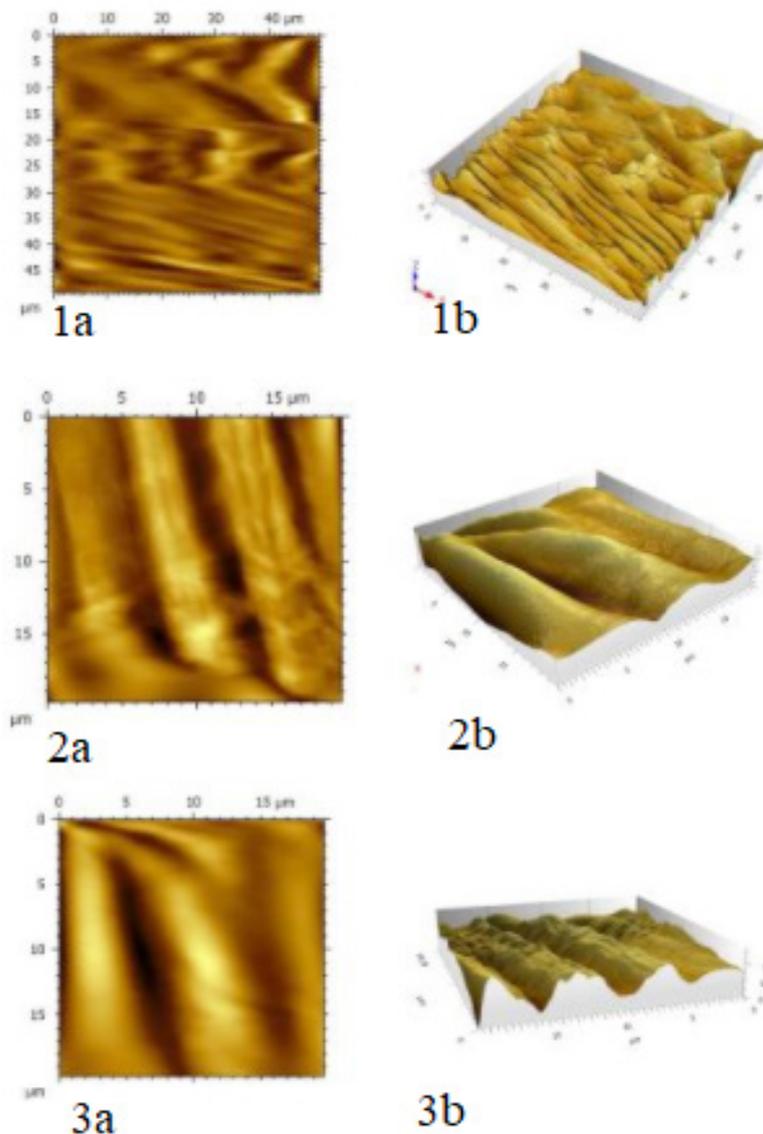


Figure 1a and 1b. AFM image of enamel surface treated with coca cola showing highest demineralization.

Figure 2a and 2b. AFM image of enamel surface treated with wine showing moderate demineralization.

Figure 3a and 3b. AFM image of enamel surface treated with green tea showing lowest demineralization.

Discussion

Dental erosion has shown an increased incidence in adolescents, especially after consumption of acidic beverages such as alcoholic & non alcoholic beverages [15].

Epidemiological studies have shown carbonated and non-carbonated beverages cause erosion. Hence, a comparative evaluation of the effect of alcoholic and non-alcoholic beverages on tooth enamel surface pre-treated with β -tricalcium phosphate, bioactive glass and amine fluoride was done.

The results of this study showed that all the remineralizing agents were able to provide protective effect against erosive enamel loss. Specimens pre-treated with bioactive glass showed better protective effect than the specimens pretreated with β -TCP and amine fluoride.

The lowest surface roughness value of bioactive glass (BAG) denotes it has the highest remineralization potential. This could be attributed to its ability to act as a biomimetic mineralizer, matching the body's own mineralizing traits and the formation of calcium phosphate at the surface of bioactive glass (BAG). Also, this

material was originally developed as a bone regenerative material as it has been shown to be effective at physically occluding the dentinal tubules through the development of a hydroxyapatite-like mineral layer [16].

Gjorgievska et al. determined the remineralizing effects of BAG on bleached enamel and concluded that BAG deposits were found on the enamel surface of all the specimens, demonstrating that they may act as a reservoir of ions available for remineralization at sites of possible demineralization [17].

β -TCP, showed decrease in resistance to demineralization in comparison to BAG. This can be credited to the formation of stabilized amorphous calcium fluoride phosphate phase. The smaller and amorphous deposits formed by β -TCP, while larger and more angular deposits formed by BAG explains the less remineralization potential of β -TCP in comparison to BAG [16].

Enafix is a complex mixture of calcium sucrose phosphate and amine fluoride with inorganic ACP. During brushing, calcium sucrose phosphate (anticay) in Enafix breaks down and releases calcium, phosphate, and sucrose phosphate ions into the saliva. Calcium and phosphate ions attach onto the enamel surface, decrease the rate of enamel solubility and increase the rate of remineralization.

The added factor – sucrose phosphate anions – adsorbed onto the surface of tooth reduced the rate of acid dissolution [18]. However, the disadvantages of this inorganic fluoride in comparison to organic fluoride present in Enafix are that it basically forms a thick layer of calcium fluoride by reaction with hydroxyapatite of enamel. Hellwig et al. found that due to this thick layer of calcium fluoride formed, there is lower bioavailability of fluoride ions. This could possibly be the reason of Enafix having the least remineralization potential value when compared to Bioactive glass and β -TCP [19].

Coca cola was used in this study to induce artificial erosive effect as in other studies since it is a commonly consumed acidic beverages amongst the youngsters.

Wine was used in this study because its acidity may have a deleterious effect on teeth. With changes in lifestyles, the consumption of wine has increased. The pH of wine has been reported to range from 3.0 to 3.8 which makes it a serious player in dental erosion.

Green tea is the second non-alcoholic beverage used in this study because of its numerous health benefits and increased consumption worldwide. Few *in situ* studies have shown that green tea and a rinse containing green tea extract were able to reduce erosive dentin wear [20,21].

These products are rich in polyphenols and inhibit the activity of metalloproteinases. It has been suggested that MMPs could be used to control the loss of the dentin matrix. However, MMPs are released as inactive precursor, requiring activation by a low pH to degrade extracellular

matrix components. Therefore, the demineralization process is increased by the action of acids on the organic matrix of dentin and the activation of the MMPs [22].

To reduce this process, green tea has a significant inhibitory effect on MMPs and dentin demineralization.

The results of this study shows that Coca Cola had the highest erosive potential when compared to wine and green tea. These results are similar with literature data which shows that cola-based drinks have much higher erosive potential than wine, orange juices and green tea [23,24].

According to information from the manufacturers, Coca-Cola contains phosphoric acid which results in a very low pH value of 2.67 which explains the high erosive effect of coca cola compared to wine and green tea [25].

The results show no significant difference between wine and coca cola in all the groups. This can be attributed to low pH of wine which ranges from 3.0 to 3.8, with white wine being slightly more acidic than red wine. Wine contains tartaric, malic acids and smaller concentrations of citric and succinic acids which imparts acidity. Saliva is normally supersaturated with calcium and phosphate supersaturated in relation to enamel hydroxyapatite. Acidic environment results in undersaturation of these salivary salts with softening of dental enamel. The altered enamel now becomes susceptible to wearing away by masticatory forces and tooth brushing [26].

The demineralization potential values of green tea were least when compared to wine and coca cola owing to its antioxidant property.

Demineralization of the tooth is characterized by initial softening of the enamel surface. This process is followed by continuous dissolution of the enamel crystals, resulting in permanent loss of tooth volume with a softened layer at the surface [27]. The dentin gets continuously exposed in advanced stages and exposes the organic matrix to breakdown by matrix metalloproteinases (MMPs) present in dentin and saliva. MMPs play a major role in chemical degradation of the organic matrix and in the progression of dentin erosion [28].

Green tea minimizes this process because it has a significant inhibitory effect on MMPs and dentin demineralization.

In this study, samples immersed in wine, coca cola and green tea showed minimum surface roughness values when treated with bioactive glass followed by β -TCP and amine fluoride.

The results are in concordance to a study conducted by Chintan Joshi et al. who compared the remineralization potential of bioactive glass (BAG) Novamin (SHY-NM) and functionalized tricalcium phosphate (f-TCP) (Clinpro Tooth Crème) and showed that BAG Novamin (SHY-NM) showed the maximum remineralization potential than by

f-TCP (Clinpro Tooth Creme) [16].

Alaudin and Fontana studied the remineralization efficiency of Novamin and fluoride using confocal laser scanning microscopy and stated that Novamin dentifrice produced significantly more remineralization than did the fluoride [29].

The results of the present study showed that among the remineralizing agents used bioactive glass was found to be highly effective and showed better preventive effect when compared to amine fluoride and β -tri calcium phosphate.

Conclusions

The present study concluded that all remineralizing agents tested were found to be effective in inhibiting the demineralization caused by alcoholic and non alcoholic beverages. Among the remineralizing agents tested, bioactive glass was found to be more effective than β -Tri calcium phosphate and amine fluoride. Among the demineralizing agents used in this study, the demineralization potential of Coca Cola was found to be highest, followed by wine and green tea pre-treated with β -tricalcium phosphate, bioactive glass and amine fluoride. Atomic Force Microscopy was found to be an efficient way to quantitatively assess the changes in surface roughness of enamel. However, future research is suggested to validate the results obtained in the present study.

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