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REMINERALIZATION POTENTIAL USING CALCIUM SUCROSE PHOSPHATE (ENAFIX) ON ARTIFICIAL CARIOUS LESION – A POLAROID MICROSCOPIC STUDY

KEYWORDS: ENAFIX , calcium sucrose phosphate , remineralization

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ABSTRACT *Introduction: Prevention of initiation and interruption in progression of early lesions are the desirable modes of caries management.*

Objective: The aim of this study was to evaluate the remineralization potential of calcium sucrose phosphate(ENAFIX) on artificially demineralized human enamel using polarized microscopic study.

Materials and methods: The study group consisted of 20 sound human mandibular premolars subjected to demineralization in a demineralizing solution for 10 days followed by remineralization with application of ENAFIX toothpaste and placed in artificial saliva for 10 days . The teeth were sectioned and visualized under polarized microscope and the mean depth of penetration of lesion before and after remineralization was calculated.

Results: Statistical analysis was done using paired t test. The mean depth of penetration of artificial carious lesion was 147 µm and after remineralization it was seen to be 141 µm , the study showed significant difference (p<0.001)

Conclusion: It can be concluded that twice a day periodic application of ENAFIX tooth paste could significantly reduce the depth of enamel lesion produced subsequently by acid challenge.

INTRODUCTION

Dental caries has high prevalence and it is the second most common disease throughout the world , inspite of advances in field of dentistry and shifting focus primarily on preventive dentistry . Prevention of initiation and interruption in progression of early lesions are the desirable modes of caries management . The concept of tooth demineralization is undergoing a paradigm shift where the treatment is now directed toward reverting the process to near normalcy . There has been an explosion of interest in technologies that may have value for remineralization of enamel.[1]

OBJECTIVE

To evaluate the remineralization potential of calcium sucrose phosphate (ENAFIX) on artificially demineralized human enamel on polarized light microscopy.

MATERIAL AND METHODS

An in vitro study was designed and conducted in the Department of Paedodontics and Preventive dentistry , Yenepoya Dental College with the assistance from Department of Oral Pathology , AB Shetty Memorial Institute Of Dental Sciences. Ethical clearance for the study was obtained from ethical committee.

Twenty sound extracted human mandibular premolars were cleansed of soft tissue debris and inspected for cracks , hypoplasia and white spot lesions . The teeth were then coated with a nail varnish , leaving a narrow window , approximately 3 x 3 mm wide , on the sound , intact surface of the buccal and lingual surface of enamel . Each tooth was subsequently immersed in the demineralizing solution (2.2 mM CaCl , 2.2 mM KH_2PO_4, 0.05 M acetic acid having pH adjusted to 4.5 and 1 M KOH) for 10 days to produce artificial lesions . The teeth were checked for demineralization using laser fluorescence (DIAGNOdent) . The ENAFIX toothpaste was applied onto the buccal window with an applicator tip for 2 minutes twice daily for 10 days and placed in artificial saliva . After which the nail polish was carefully removed from the specimens . Ground sections were revisualized under polarized light microscopy . After imbibition of the sections in water, polarized light microscopy (PLM) was employed to qualitatively evaluate the body of the lesions in each of the enamel sections . Depth of lesions was measured using Motic software.

RESULTS

The study was conducted on 20 samples and after subjecting to demineralization and remineralization , the samples were cross sectioned and seen under polaroid microscope . The following images were obtained-

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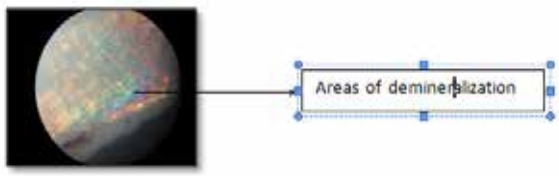

Fig (a) After demineralization-

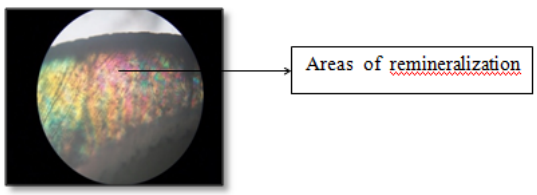

Fig (b) After remineralization using ENAFIX toothpaste

DEPTH OF PENETRATION

Fig (c) After Demineralization

Fig (d) After Remineralization with ENAFIX

STATISTICAL ANALYSIS

The statistical analysis was done using paired t test and the following values were obtained.

Table 1

		Mean(µm)	N	Std. Deviation
Pair 1	Demineralized	147.5200	20	11.50984
	Remineralized	141.8000	20	13.50025

Table 2

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Demineralized and Remineralized	5.72000	5.65170	1.26376	3.07492	8.36508	4.526	19	.000

INFERENCE

Table 1 - The mean depth of penetration after demineralization and remineralization was 147.5 µm and 141.8 µm respectively.

Table 2 - Demineralized and remineralized mean depth of penetration values , significant differences were seen.

Table 3 - Paired mean difference between the demineralized and remineralized samples is 5.72 with standard deviation is 5.65 with high significance (p < 0.001).

DISCUSSION

The recent approach in caries management is the non invasive method . Non – cavitated and cavitated lesions extending up to dentinoenamel junction can be arrested if the cariogenic challenges of certain micro-environment are sufficiently controlled and if therapeutic agents are applied for tissue healing.[1]

Group Pharmaceuticals Ltd . has specially formulated a toothpaste Enafix - CaSP using Anticay® technology in India . The anticay in Enafix is Calcium sucrose phosphate which quickly breaks down and releases calcium , phosphate and sucrose ions . According to the manufacturers , it has the common ion effect wherein the rate of remineralization increases , it not only remineralizes the surface enamel but also at depth.

Dental caries is a multi-factorial disease that involves the interaction between diet , dental plaque containing bacteria , and host factors , such as tooth surface , saliva , and the acquired pellicle (Zero, 1999) . Dental caries is initiated via demineralization of tooth mineral by organic acids . The organic acids are produced by plaque bacteria following exposure to fermentable carbohydrates . When a critical pH is reached , the organic acids are able to diffuse into the enamel surface through the acquired pellicle , commencing demineralization.

During demineralization , less soluble phases of dicalcium phosphate dihydrate (CaHPO4.2H2O) and fluoridated hydroxyapatite ($Ca_5(PO4)_3(OH)$) precipitate out of the enamel . This process continues until equilibrium is achieved between the enamel and the oral environment . Demineralization can continue as long as the oral pH remains acidic (Margolis and Moreno , 1990).

When the oral pH rises above the acidic level , the remineralization process of the tooth surface can begin . Calcium and phosphate , which are present in the saliva , diffuse into the enamel with the help of fluoride to remineralize crystalline structures in demineralized areas . The rebuilt structures consist of fluoridated hydroxylapatite and fluorapatite , which are much more resistant to acid attack than the original structure (Selwitz et al , 2007) . The processes of demineralization and remineralization occur several times throughout the day and if balanced , will not result in carious lesions . However , if the balance is tipped more towards demineralization rather than remineralization , the lesion will progress and eventually become a frank cavitation (Featherstone , 2000).

Mode of action of ENAFIX

- Anticay® in enafix – Quickly breaks down and releases calcium , phosphate and sucrose phosphate ions into the saliva . Anticay® is a mixture of calcium salts of sucrose phosphate esters , complexed with inorganic calcium orthophosphate . It is composed of 10-12% calcium and 8 - 10% phosphorous by weight . Calcium and phosphate in aque-

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ous media tend to form insoluble precipitates . An important feature of Anticay® is that it allows for the creation of aqueous solutions containing very high concentrations of calcium and phosphate without precipitation occurring The widespread influence of calcium and phosphate in biological systems means that this simple property of solubility makes Anticay® extremely useful in a large number of therapeutic settings . Anticay acts as an ideal carrier for calcium and phosphate in water .

- Adsorption of ions - These ions rapidly adsorbs on enamel surface .
- Inhibits demineralization - Sucrose phosphate ion - decreases rate of acid dissolution of hydroxyapatite
- Rapid remineralization – Calcium & Phosphate ion – increase rate of remineralization by common ion effect.

If retardation of the demineralization of tooth enamel can inhibit dental caries then it is also likely that the opposing reaction of the equilibrium , remineralization can also influence the progress of a carious lesion.[2]

The non-invasive treatment of early caries lesions by remineralization is a major advantage in clinical management and researchers have investigated the low cariogenic potential and possible cariostatic activity of dairy products such as milk , casein , caseinates , and cheeses.[3,4]

Polarized light microscopy allows visualization of enamel demineralization and remineralization and is often used to evaluate carious lesions .

A polarized light microscope consists of a light microscope plus a polarizer and analyzer set perpendicular to one another . Both are made of prisms of calcite or a sheet of Polaroid , which transmit light oscillation in one plane (Weyrich , 1994) . When a sample is placed between the polarizer and the analyzer , the sample modifies the plane of light and produces a series of interference colors.

Hydroxyapatite is a uniaxial anisotropic crystal i.e. it has one optic axis that is coincident with its crystal axis . Hydroxyapatite is also birefringent , splitting a light ray into two components , which travel at different velocities and are polarized at right angle to one another , releasing different color and light intensities (Weyrich , 1994) . A material that consists of a non-cubic crystal is given a sign of birefringence determined by the velocity of its resultant rays . Enamel is composed mostly of inorganic hydroxyapatite (-) with a small amount of organic material (+) interspersed.[5]

Polarized light microscopic analysis was chosen in the current study since it is a technique extremely sensitive to changes in hard tissues . With respect to de- and remineralization , it can qualitatively show the areas of mineral loss and mineral gain represented by the visualization of areas with different porosities and birefringence.[6]

Areas of demineralization were seen as green in color in the images seen under polaroid microscopy and pink color were areas of remineralization . Depth of penetration of the artificial lesion was 147.5 µm and after remineralization it was seen to be 141.8 µm.

There are very few studies done on remineralization potential of calcium sucrose phosphate using polaroid light microscopy . Hence this study was done to determine the potential role of ENAFIX on management of initial caries lesion and from our study it was seen that when ENAFIX was applied , the increase in remineralization and decrease in lesion depth were seen.

Conclusion

From the present study , it can be concluded that periodic application of ENAFIX tooth paste could significantly reduce the depth of enamel lesion produced subsequently by acid challenge.

The conclusions drawn in the present in vitro study need to be substantiated by longitudinal caries incidence studies and in situ studies with respect to calcium sucrose phosphate incorporated paste .

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