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"Grapple before they trample" – Relative analysis of potential efficacy in remineralisation of primary tooth enamel by non-fluoridated dentifrices –An *In vitro* study

("Lucha antes de que te pisoteen": análisis comparativo de la eficacia potencial de los dentífricos sin flúor en la remineralización del esmalte dental primario: un estudio in vitro)

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Abstract (english)

S-ECC major oral health concern globally. This has led to major change in lifestyle and customs of people; modernization brought lot changes in daily routine leading to increase in rates. This caries process has continuum from first dynamic changes in hydroxyapatite crystals to visible white-spot lesion. This brings a need to prevent hydroxyapatite from modifying its structure. For years fluorides have been used for caries prevention. The major shortcoming is ability of enamel remineralisation, limited by the low concentration of calcium and phosphate ions in saliva. The current study used non-fluoridated materials that provide additional ions in oral environment for remineralisation and also avoid fluoride toxicity in infants/toddlers, thus supporting the concept of PREVENTION than RESTORATION. 30 (N=30) therapeutically extracted primary maxillary central incisors were sterilised and stored in artificial saliva until processed. After grouping as group-I(n=10) ToothMin, group II(n=10) negative control and group-III(n=10) EnaFix, subjected to cyclic demineralisation and remineralisation(once every 24hours for 10days). Each sample was grounded separately, to estimate calcium and phosphorous weight percentage using EDX technique and confirmed by SEM. The weight percentage of calcium-phosphate in hydroxyapatite estimated higher in the EnaFix than ToothMin indicating; EnaFix shows higher remineralisation in given time, because of action of substantivity and anti-plaque adherent. In conclusion, comparing results even though there is no much difference in statistical measure, EnaFix has better hydroxyapatites than ToothMin. The crystals are formed at higher rate in EnaFix, though not similar to the natural enamel prism in tooth.

Keywords(english)

ToothMin; demineralisation*;* enamel remineralization, *EnaFix; tooth*

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Resumen(español)

La caries dental de la primera infancia (S-ECC) es una preocupación importante para la salud bucal a nivel mundial. Esto ha provocado cambios importantes en el estilo de vida y las costumbres de las personas; la modernización trajo consigo muchos cambios en la rutina diaria, lo que ha llevado a un aumento en las tasas. Este proceso de caries tiene un continuo desde los primeros cambios dinámicos en los cristales de hidroxiapatita hasta la lesión visible de mancha blanca. Esto genera la necesidad de prevenir que la hidroxiapatita modifique su estructura. Durante años, se han utilizado fluoruros para la prevención de la caries. La principal deficiencia es la capacidad de remineralización del esmalte, limitada por la baja concentración de iones de calcio y fosfato en la saliva. El presente estudio utilizó materiales no fluorados que proporcionan iones adicionales en el entorno oral para la remineralización y también evitan la toxicidad del fluoruro en bebés/niños pequeños, apoyando así el concepto de PREVENCIÓN en lugar de RESTAURACIÓN. Treinta (N=30) incisivos centrales maxilares primarios extraídos terapéuticamente fueron esterilizados y almacenados en saliva artificial hasta su procesamiento. Después de agrupar como grupo-I (n=10) ToothMin, grupo II (n=10) control negativo y grupo-III (n=10) EnaFix, sometidos a desmineralización y remineralización cíclica (una vez cada 24 horas durante 10 días). Cada muestra se molió por separado, para estimar el porcentaje en peso de calcio y fósforo usando la técnica EDX y confirmado por SEM. El porcentaje en peso de fosfato de calcio en hidroxiapatita estimado mayor en EnaFix que en ToothMin, lo que indica que EnaFix muestra una mayor remineralización en un tiempo dado, debido a la acción de la sustantividad y la adherencia antiplaca. En conclusión, comparando los resultados, aunque no hay mucha diferencia en la medida estadística, EnaFix tiene mejores hidroxiapatitas que ToothMin. Los cristales se forman a una tasa más alta en EnaFix, aunque no es similar al prisma de esmalte natural en el diente.

Palabras clave(español)

ToothMin; desmineralización; remineralización del esmalte, EnaFix; diente.

Introduction

Dental caries is a localized chemical dissolution of the tooth surface caused by metabolic events taking place in the biofilm covering the affected area. A shift in the ecology and metabolic activity of the biofilm can cause an imbalance in the equilibrium in the form of pH fluctuations. The shift in the pH can influence the chemical composition of the tooth structure. Hydroxyapatite is the main component of enamel (95%) and dentine (75%) (1). The enamel surface is in a state of dynamic equanimity with its surrounding environment (2). When the pH in the oral cavity drops, the acidic environment leads to increased solubility of the apatite crystals increases resulting in demineralization, when the pH increases, the alkaline environment favors remineralization (3). When the demineralization phase prolongs, excessive loss of mineral ions leads to white spot lesion (WSL), early, initial or incipient lesion, progressing further to form cavitation and total destruction of the tooth. As dental caries is a slow process, during early stages non-invasive interventions can convert the initial lesion from an active to inactive state (4). Restorative procedures form 71% of all treatments for dental caries, with recurrent caries poses a looming threat (5.) Thus the concept of minimal intervention dentistry has changed the perspective of caries management, from “extension for prevention,” as proposed by GV Black. The goal of modern dentistry is to manage non-cavitated carious lesions non-invasively through remineralization in an attempt to prevent disease progression, and to improve strength, esthetics and function of teeth (6). Diagnosis of carious lesions at earlier stages and its remineralization has led to new era in the modern preventive dentistry (7). Remineralization is defined as the process whereby calcium and phosphate ions are supplied from a source external to the tooth to promote ion deposition into crystal voids in demineralized enamel, to produce net mineral gain (8).

Fluoride has been used as a gold standard treatment for remineralizing initial enamel carious lesion (9). However, there was a low concentration of calcium (Ca++) and phosphate (PO4)2- ions in saliva after using fluoride dentifrice and high levels of fluorides lead to toxic effects. Anticay, a CaSP–calcium orthophosphate complex, supplies both calcium and phosphate in a soluble form. It is a fine, white, nonhygroscopic powder with a neutral blend taste. It contains approximately 11.5% calcium on a dry weight basis. This complex helps reduce the acid solubility of enamel and increase the rate of remineralization by a common ion effect (10, 11). This anticay technology has been incorporated into toothpastes such as ToothMin (Abbott Healthcare) and EnaFix (Group Pharmaceuticals Ltd., India). Effective anticariogenic and remineralizing agent which delivers the ions directly to target area and relatively safe if consumed by lesser age group. There is a paucity of data regarding the efficacy of ToothMin and

EnaFix, hence in this in-vitro study, we examined the effect of remineralisation of ToothMin and EnaFix on maxillary primary central incisor enamel remineralization through the analysis of Energy Dispense X-Ray Beam (EDX) and scanning electron microscopy (SEM) images.

Materials and methods

In this study, 30 extracted maxillary primary anterior teeth (N=30) were sectioned into dimension of 3*3 mm each, then randomly divided into three groups.

- *Group I* - ToothMin (n=10),
- *Group II* - Negative control (n=10),
- *Group III* - EnaFix (n=10).

Inclusion criteria

- Extracted first and second deciduous incisors

Exclusion criteria

- Teeth with developmental defects
- Teeth with caries and white spot lesion
- Teeth subjected to restorative treatment

The prepared teeth samples were stored in the artificial saliva (pH-7.2) freshly prepared based on McKnight Hane and Whitfort formula (1991) (12) each time when not in processing. Demineralization was carried out with freshly prepared demineralising agent HCl + H2O2. Each tooth sample were subjected to 15 cycles of 30seconds in 10 minutes in the demineralizing agent and then treated with water. Remineralisation agent, (group I – ToothMin, group II –control and group III – EnaFix) was applied for 3 minutes every day once for 7days.

After 7 days, the tooth were sectioned into two using a power saw and processed under for energy dispersive X-ray (EDX) (Thermo Noran, Thermo Scientific, Rockford, IL, USA) analysis to determine the elemental levels of (Ca++) and phosphate (PO4)2- ions. Once the calcium and phosphate ions were calculated, the enamel specimens were then placed on a metal mounting block and then kept inside the gold sputter coater (Q150R, Quorum technologies, UK) for examining the surface characteristics. After sputtering the specimens were observed under SEM (GeminiSEM, Zeiss microscopy, Germany) at ×5,000 and ×10,000 magnifications at 15kv.

Statistical analysis. Was performed by using IBM SPSS Statistics for Windows, (Version 22.0. Armonk, NY: IBM Corp). The normality of data was analyzed using Kolmogorov-Smirnov test and the data followed normal distribution. One-way analysis of variance (ANOVA) test was used to test the mean differences between the groups. Criterion for statistical significance was set at $p<0.05$.

Results

The weight percentage of calcium in hydroxyapatite given in table 1 and figure 1, was significantly higher in tooth specimens treated with EnaFix 4.582 ± 1.11 compared to ToothMin 3.771 ± 0.73 ($p< 0.05$), while phosphate ions were also higher in ToothMin 1.653 ± 0.87 when compared to EnaFix 1.102 ± 0.29 ($p < 0.05$)

On SEM observation, Pre mineralization showing uniform smoothness and enamel pattern with evident incremental enamel lines (figure 2). SEM image of the enamel surface post demineralization (Figure 3), the enamel rods were collapsed due to lack of orientation of the hydroxyapatite crystals and with no the fish scale appearance. The enamel surface appeared rough and uneven, and increased porosities were observed. The configuration of sound enamel topography is barely apparent with porous defects, the enamel rods are barely discernable with evident porosities with Open dentinal tubules are seen (Figure 4).

Table. 1. Comparison of weight % of calcium and phosphate ions between EnaFix and ToothMin.

	Remineralization Agent	Mean ± SD	p-value
Calcium	Tooth min	3.771 ± 0.73	0.00
	Negative control	0.430 ± 0.54	
	Enafix	4.581 ± 1.11	
Phosphate	Tooth min	1.653 ± 0.87	0.00
	Negative control	0.020 ± 0.47	
	Enafix	1.102 ± 0.29	

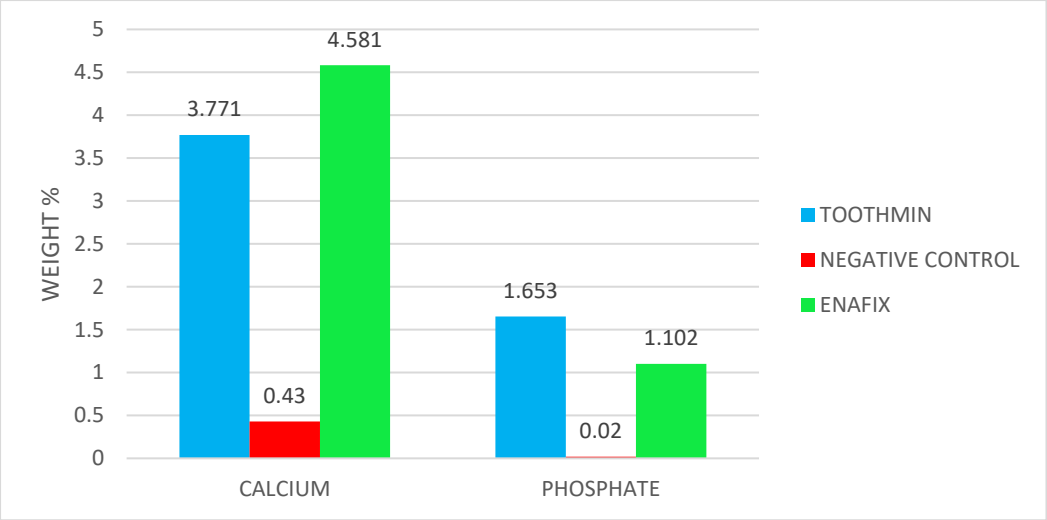


Figure 1. Comparison of weight % of calcium and phosphate ions between EnaFix and ToothMin

After treatment with the remineralizing agent (Figure 5a and 5b). All the test groups revealed a layer of surface deposition of minerals obliterating the defects, filling up the rods and interrod region, showing an uneven surface compared to the control group. Certain areas of calcifications were evident. Areas of mineralized deposits are discernible and are seen profusely scattered along the porous defects.

Discussion

Dental caries is a multi-factorial disease process caused by acidic environment in the oral cavity contributed by acids, from bacterial metabolism which results in dissolution and demineratlization of the enamel matrix. Demineralization starts at a critical pH (5.5) of hydroxyapatite, the oral environment is undersaturated with mineral ions (13). The concept of tooth demineralization has undergone fundamental changes, reverting the tooth to normalcy through remineralization is given emphasis compared to excavation followed by placement of a restorative material (14). The duration and frequency of the acidic contact on the tooth surface is a crucial factor in determining the level of destruction such as incipient white lesions to cavitations on the tooth structure, when the surface remains partially intact, there is a high probability of arresting or even reversing the lesion (15).

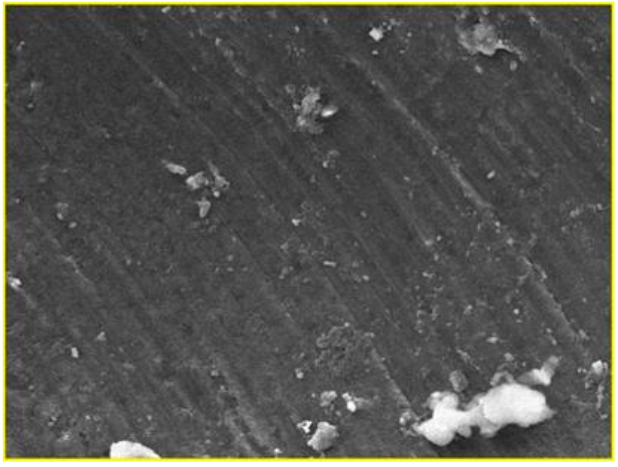


Figure 2. Pre demineralization sem 1000x

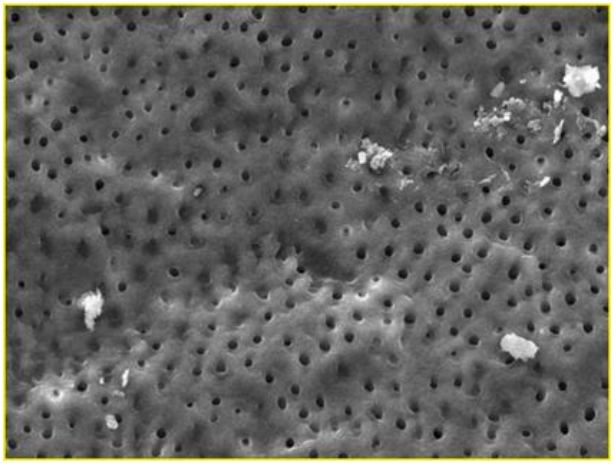


Figure 3. Post-demineralization sem 1000x

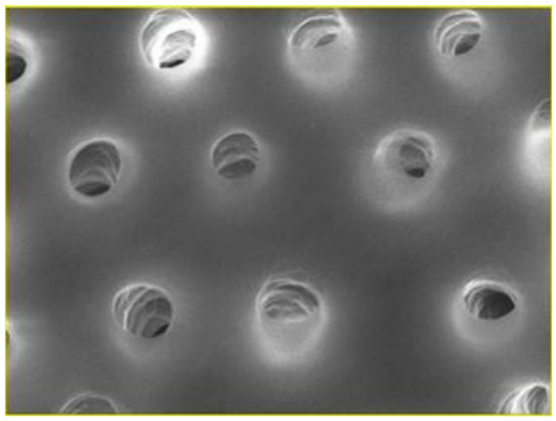


Figure 4. Post demineralization inset 5000x

Remineralization helps in regaining the lost calcium, phosphate, and fluoride ions from the enamel surface and is replenished through fluorapatite crystals, which are impervious to acidic dissolution. Though remineralization has been a major area of investigation, it is still difficult to exactly define the efficacy of various remineralization methods. Until today, fluoride therapy has remained the most popular of all remineralization methods, routinely fluoride dentrifices containing 1000 ppm of fluoride are recommended for all age groups (16). However, the use of greater amount may increase the risk for fluorosis, especially among children <6 years of age (17). Hence, nonfluoride-containing dentifrices with remineralizing agents have been developed. Anticay systems, which is a mixture of calcium sucrose phosphate with inorganic amorphous calcium phosphate are commercially available.

Calcium Sucrose Phosphate complex (AntiCay) acts as a reservoir of calcium phosphate ions which rapidly breaks down and releases calcium, phosphate and sucrose phosphate ions into the saliva. The ions, rapidly adsorbs onto the enamel, decrease the rate of enamel solubility under acidic conditions & at neutral pH increase the rate of remineralization. The added factor- sucrose phosphate anions adsorbed onto the enamel surface decrease the rate of acid dissolution. Thus EnaFix helps remineralize and inhibit dental caries. It also exhibits increased substantivity and the presence of ascesulflame gives the toothpaste Anti plaque adherent property (19). In our study, EnaFix showed higher remineralisation in given time, because of action of substantivity and anti-plaque adherent property. This finding was similar with the results of the study done by Titty TM et al (20) and Menon LU et al (21) who demonstrated significant remineralization post usage of such AntiCay products. The present study showed that demineralized enamel would not only regain its original value but also showed an increase in mineral content after the remineralization procedure using both EnaFix and ToothMin.

Remineralization results in a change in the surface roughness, morphology, and the mineral content of enamel, these parameters can be used to determine the extent of remineralization using EDX and SEM imaging. This helps in quantification of various elements like calcium, phosphorus, fluoride, magnesium and sodium in both atomic and weight percentage (21). In the present study, the changes in the topography was evident however, it was to a great extent and sizes of the white spots were different compared to the grey levels were different at week six among the groups. It has been shown that enamel remineralization is evident in the first 3– 6 weeks, and it then progresses slowly and steadily after 12 weeks, this finding was concurrent with the findings of the study by Shaik ZA et al (22) and Nanwal R et al (23). Comparing all results, in the present study, EnaFix seems to be a potent remineralizing agent considering

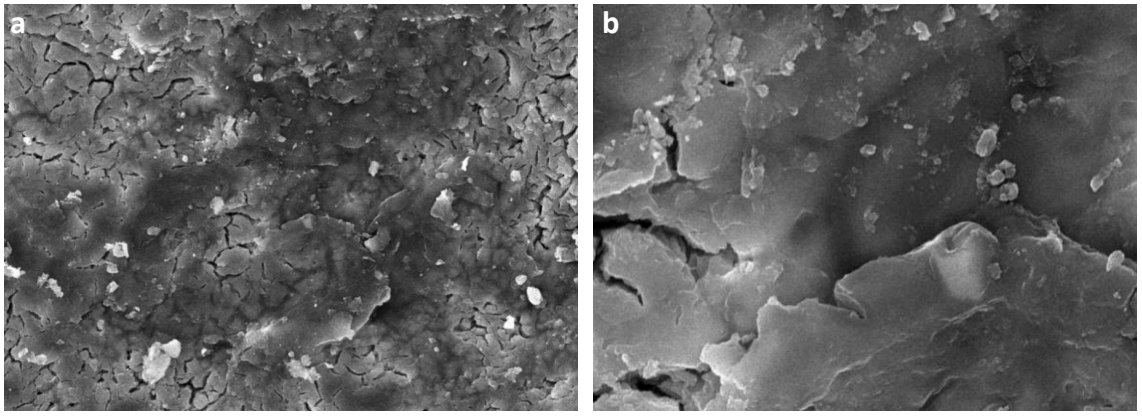


Figure 5. 5a. Post remineralization 1000X. 5b. Post remineralization 5000X

all the properties described. Thus, components of this toothpaste could be used with other regularly used dentifrices to impart its beneficial effects and improve the quality of the toothpaste

Limitations of this study include, lack of histopathological analysis along with EDX and SEM analysis of the samples, would give better insights into remineralization potential. Since the study is limited to in vitro, it may be different when compared to the in vivo with dynamic biological system, further clinical trials with large sample size are necessary to assess the remineralization potential and longevity.

In conclusion, EnaFix has better hydroxyapatites than ToothMin. The crystals are formed at higher rate in EnaFix, though not similar to the natural enamel prism in tooth. Parents along with the children should be educated about the twice brushing per day with appropriate selection of toothpaste preferably non fluoridated is recommended for all the ages.

Abbreviations

WSL - white spot lesion
Ca++ - Calcium ions
(PO4)2- Phosphate ions
EDX - Energy Dispense X-Ray Beam
SEM - Scanning electron microscopy

Conflict of interest

None to declare.

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