ORIGINAL RESEARCH PAPER

Pedodontics

KEY WORDS: White Spot lesions, Caesin Phosphopopetide-Amorphous Calcium Phosphate, Calcium Sucrose phosphate, Scanning Electron Microscope

COMPARATIVE EVALUATION OF DIFFERENT REMINERALIZING AGENTS ON ARTIFICIAL CARIES-LIKE ENAMEL LESIONS USING SCANNING ELECTRON MICROSCOPE.

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ABSTRACT

INTRODUCTION:
Treating the non-caviated lesion in non-invasive manner is the emerging trend of the daytoday life. And this research fullfills the criteria upto mark with different remineralizing agents at different intervals on the same surface of the tooth.

AIM:
To compare and evaluate the remineralizing efficacy of different calcium-phosphate and fluoride based delivery vehicles on artificial caries like enamel lesions using scanning electron microscope.

METHODS:
Twelve extracted human mandibular molars were collected. Acid resistant varnish was applied leaving four equal windows. And the specimens were immersed in demineralizing solution to create artificial lesions followed by remineralization under Artificial saliva, Remin Pro, Tooth Mousse Plus and Enafix for 4 week period, and are exposed to final acid challenge and were assessed using scanning electron microscope.

RESULT:
All the specimens on evalution in SEM showed evidence of thickening of their inter-rod substance early remineralization in Tooth Mousse Plus. After exposure to final acid challenge, Enafix group showed more resistance to dissolution.

CONCLUSION:
Tooth Mousse Plus has shown better remineralization properties while Enafix has shown better resistance to final acid challenge.

INTRODUCTION

It is widely accepted that oral and general health has greater impact in influencing the quality of life. According to the World Oral Health Report of 2003, oral diseases impede activities in school and work causing many productive hours to be lost each year, which still exists. Despite all the worldwide improvements, dental caries is still one of the major oral health problem affecting about 60% - 90% of school children and adults too.[1]

Dental caries of enamel is first observed clinically as a ''white spot lesion'' which is a small area of sub-surface demineralization. The body of the sub-surface lesion may have lost as much as 50% of its original mineral and often has an ''apparently intact surface layer''over it.2 The term remineralzation is so been defined as the process whereby calcium and phosphate ions are supplied from a source external to the tooth surface in order to promote ion deposition into the crystal voids of demineralized enamel to produce net mineral gain.[3]

The ultimate goal of modern dentistry is to manage non-cavitated carious lesions in a non-invasive manner in an attempt to prevent further disease progression and preserve integrity of healthy tooth substrate. Healing the demineralized lesions using remineralizing agent is the common way of managing non-cavitated lesions. [4]
Zero et al 2006 proposed the requirements of an ideal remineralizing agent

- Diffuses into the subsurface or else they should delivers calcium phosphate.
- Should not deliver an excess of calcium.
- It can work at an acidic pH.
- Boosts the remineralizing properties of saliva. 5

Fluoride is the most recognized remineralizing agent that interacts with oral fluids on the interface of enamel along with subsurface regions of teeth, and then combining with calcium and phosphate ions in order to form fluorapatite. The anticaries benefits of fluoride depends wholly upon the use of an effective concentration and frequency of application. There is no doubt that the discovery of the anti-cariogenic properties of fluoride was one of the most important landmarks dentistry.[6]

There is paucity in literature regarding the study of the built up mineral products stability following acid challenge. Thus, this study would compare the effect of different calcium-phosphorous and fluoride delivery systems on enamel remineralization using scanning electron microscopic analysis to completely evaluate the porous nature of demineralization.

AIM AND OBJECTIVES

AIM

The aim of the study was to compare and evaluate the remineralizing efficacy of different calcium-phosphate and fluoride based delivery vehicles on artificial caries like enamel lesions.

OBJECTIVES

- To compare the remineralizing potential of artificial saliva, Remin Pro, Tooth Mousse Plus and Enafix on artificial caries like enamel lesions using scanning electron microscopic images for qualitative assessment.
- To evaluate the better remineralization potential and ability to withstand the further demineralization potential of artificial saliva, Remin Pro, Tooth Mousse Plus and Enafix on artificial caries like enamel lesions during remineralization and after Final Acid challenge using

MATERIALS AND METHODS

SPECIMENS PREPARATION

Total of 25 extracted human mandibular molars are selected free from clinically visible abnormality were stored in an aqueous solution of saturated thymol for a period of 2 weeks. Any visible or detectable caries or any surface irregularities were excluded unless those didn't involve their buccal surface and total of 12 mandibular molars were selected for the study (Fig 1).

FIG 1: 12 MANDIBULAR MOLARS

Custom-made plastic cylindrical molds were prepared and self-cured acrylic resin was poured on each of the plastic mold. Each buccal of the mandibular molar section was horizontally mounted in acrylic resin and cured overnight.

An acid resistant nail varnish was applied using applicator tip around the exposed enamel surface on the buccal aspect of the molars, leaving four equal overtures of wax sheet which is approximately (2 mm x 2 mm) each (Fig 2). And the wax sheets are removed later once the nail varnish is set (Fig 2).

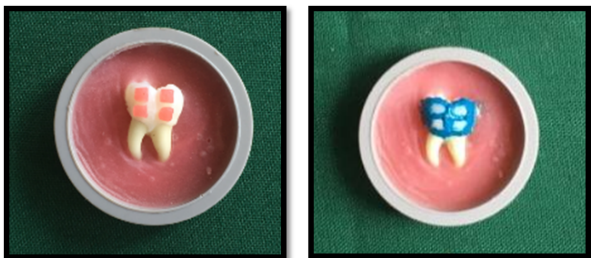


FIG 2: CREATING WINDOWS ON BUCCAL SURFACE

DEMINERALIZATION

The proposal suggested by Pulido et al 2008 10 has been followed for creating artificial caries like lesions, by individually immersing acrylic-mounted enamel specimens in continuously stirred, daily renewed demineralization solution. The mounted molars were kept in this solution for 5 days until a uniform white spot lesion were created on the surface of the window. And then the first window was coated with acid resistant nail varnish to act as control for the intial demineralization.

REMINERALIZATION

All the samples were stored in deionized water until further use. A total of twelve enamel samples embedded in acrylic slabs were produced, and were then randomly divided into four groups of 3 samples each for further remineralization.

All the specimens were randomly divided into four following groups :

GROUP 1: Artificial Saliva (Control group): No treatment was given to the enamel surface and specimens were kept in artificial saliva which was renewed every day.

GROUP 2 : Remin Pro (VOCO) – Calcium, Phosphate, Xylitol and 1,450ppm of Fluoride. (HAp – Hydroxyapatite).

GROUP 3: Tooth Mousse Plus (GC INDIA) – Caesin PhosPhopeptide Amorphous Calcium Phosphate containing 900ppm of Fluoride.

GROUP 4: Enafix (GROUP PHARMACEUTICALS) – Calcium Sucrose Phosphate and amorphous calcium phosphate.

APPLICATION OF TEST AGENTS

The samples in each group were treated with the respective remineralizing agent that were continuously applied onto the remaining 3 windows for 3 minutes once in every 24 hours for a period of 4weeks (Fig 3). Samples after application were then placed in artificial saliva prepared according to Torres et al. 201211 formula, that was changed once in every 24 hours until the final acid challenge.

Remineralization process was continued for a period of 2 weeks and the second window was coated with acid resistant nail varnish, the remaining two windows were continued remineralization and the third window was closed after a period of 4 weeks.

After these periods, the samples were reimmersed again demineralizing solution for a period of 5 days to evaluate the acid resistance of the treated surfaces and the fourth window was coated with acid reisitant nail varnish.

The 12 specimens of each group were tested by the scanning electron microscopic (EVO 18 RESEARCH; ZEISS) examination (n = 3). The nail varnish was peeled off from all of the specimens carefully before the analysis. SEM photomicrographs were captured at 1000x and 500x magnifications in order to gain their qualitative results

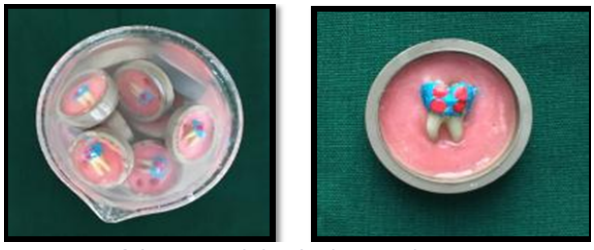
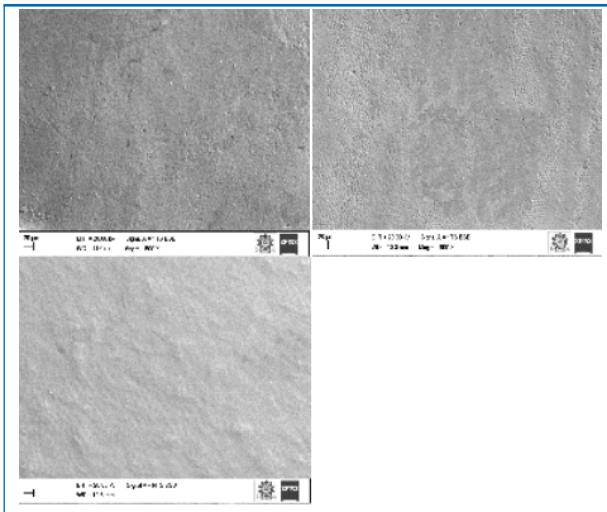


FIG 3: IMMERSION OF SAMPLES IN THE DEMINERALIZATION AGENT; FOURTH WINDOW CLOSURE

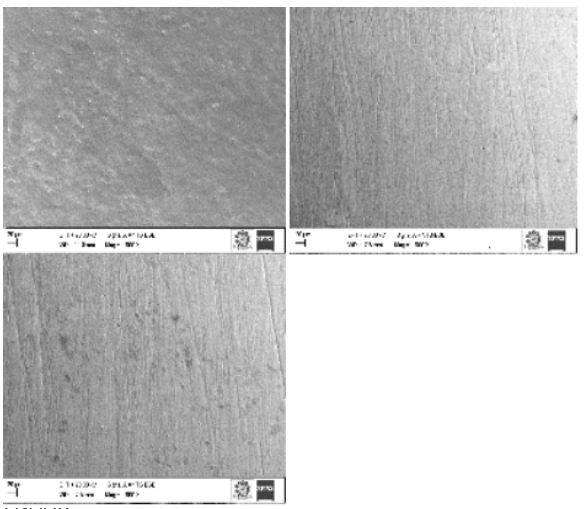
RESULTS

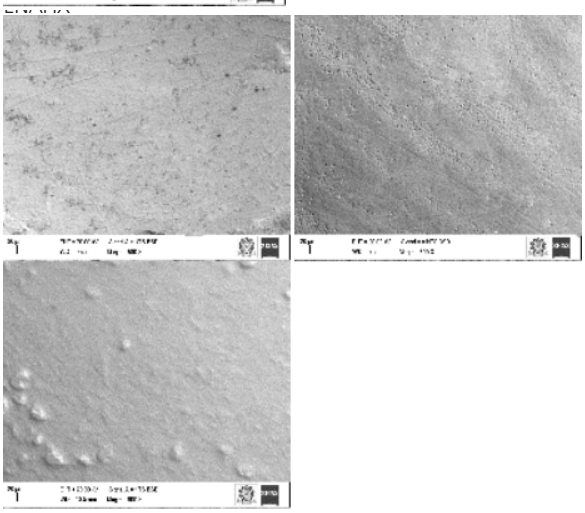
Sound enamel has homogeneous and a definitive smooth appearance. However, demineralized enamel showed a marked increase in the porosity with fish-scale pattern as depicted in SEM pictures (Fig 7). The test groups showed increased density of crystals after 2 weeks of remineralization. While after 4 weeks remineralization, enamel surfaces revealed a newer coating that progressively filled the pits and scratches where the prismatic enamel structures became hidden by the mineral deposition. All the specimens showed evidence of thickening of their inter-rod substance early by 2 week of remineralization however, extended remineralization period enhanced the growth of enamel crystals to cover over fish scale appearance of demineralized surface. In Group 2, after 4 weeks of remineralization, some of the enamel crystals were even fused together and were arranged homogenously with no obvious inter-crystalline spaces on their surface. After exposure to acid challenge, the experimental groups (Group 4, Group 3 and Group 2) showed more resistance to dissolution as compared to the control group (Group 1). The treated surfaces retained calcified deposits but with the presence of some potholes on their enamel surface

BASELINE (FIG 4: Scanning Electron Microsccopic Images)
ARTIFICIAL SALIVA

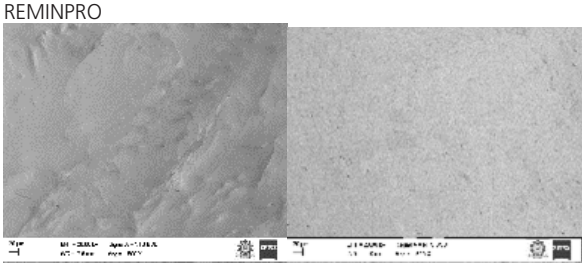


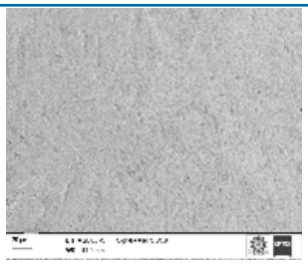
TOOTH MOUSSE PLUS





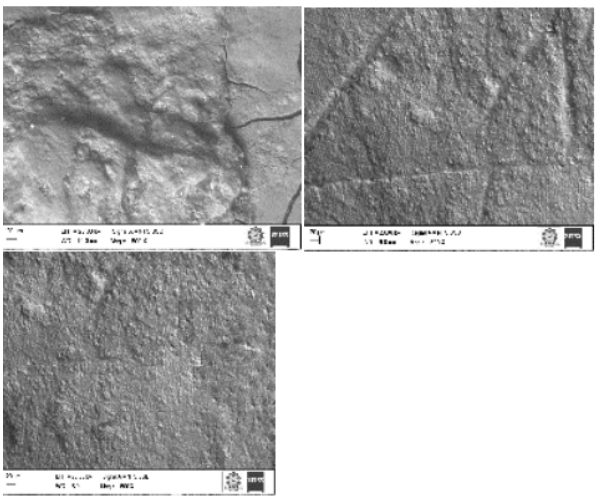
REMINPRO



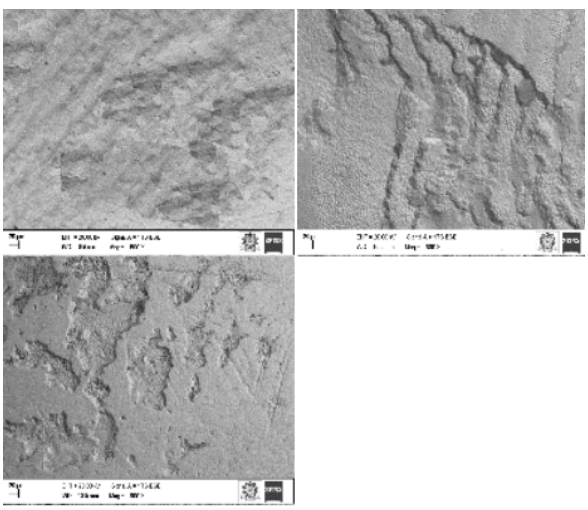


INTIAL DEMINERALIZATION

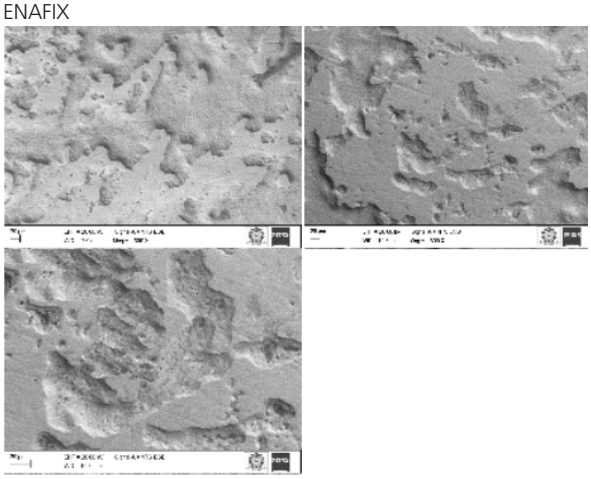
ARTIFICIAL SALIVA



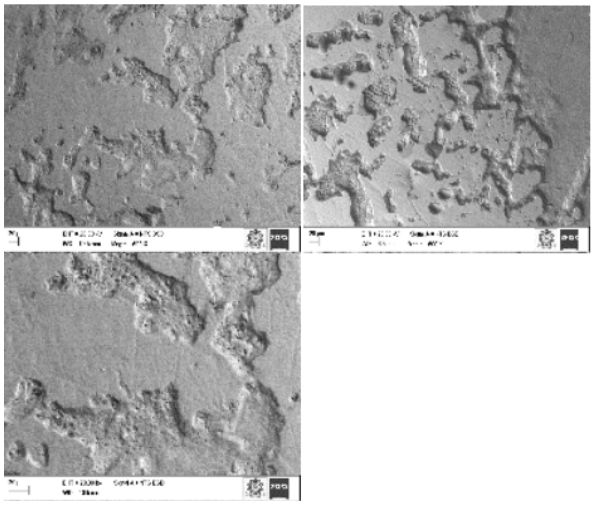
TOOTH MOUSSE PLUS



ENAFIX

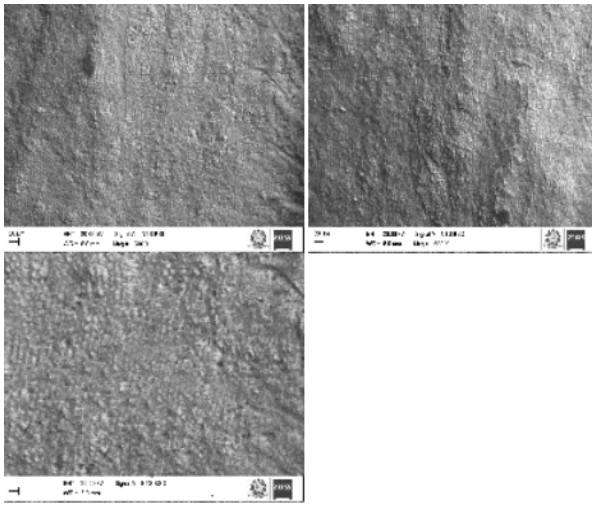


REMINPRO



2-WEEK REMINERALIZATION

ARTIFICIAL SALIVA



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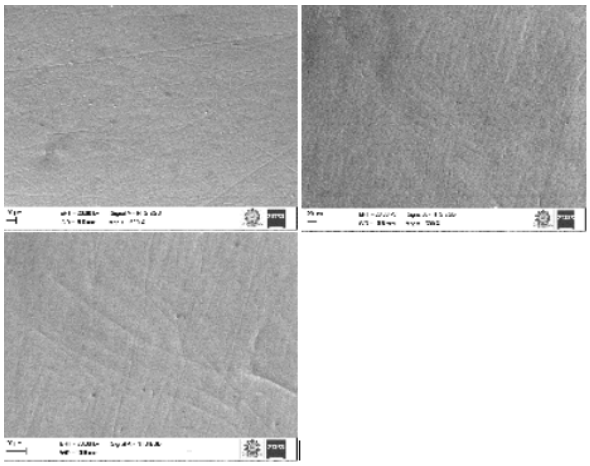

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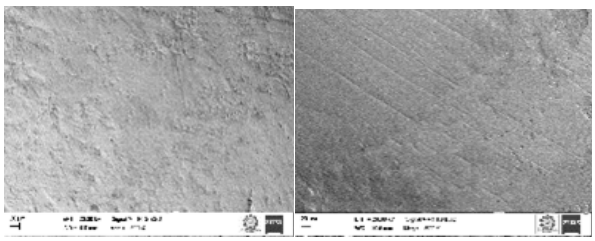
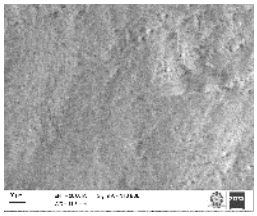
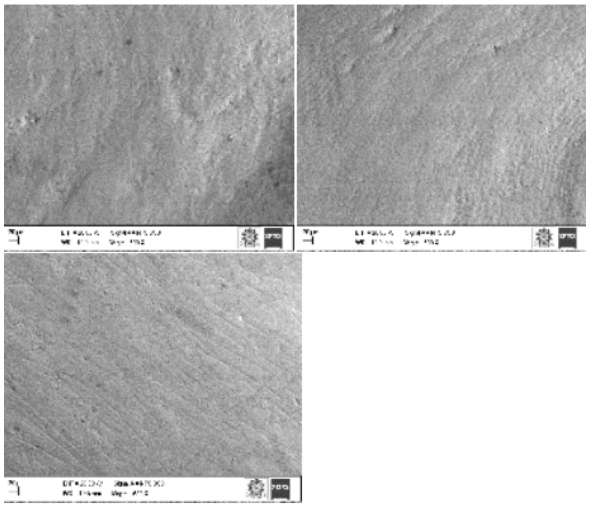


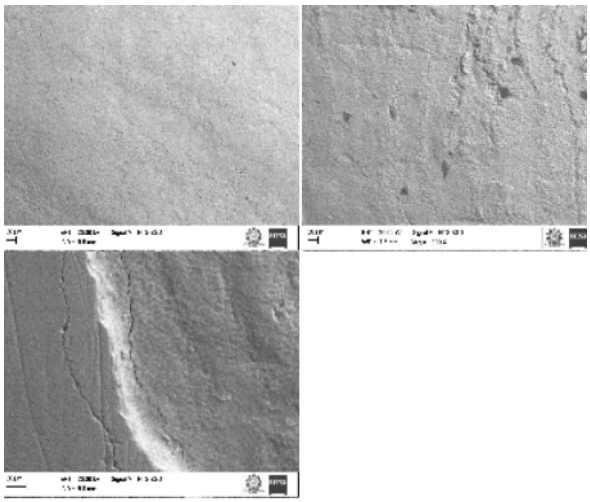


REMINPRO

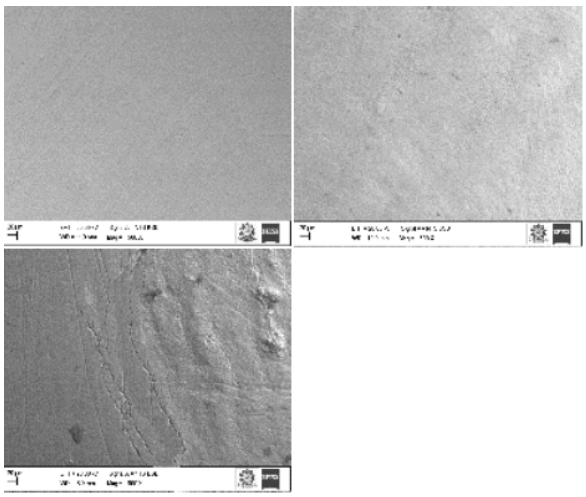


4-WEEK REMINERALIZATION

ARTIFICIAL SALIVA



TOOTH MOUSSE PLUS



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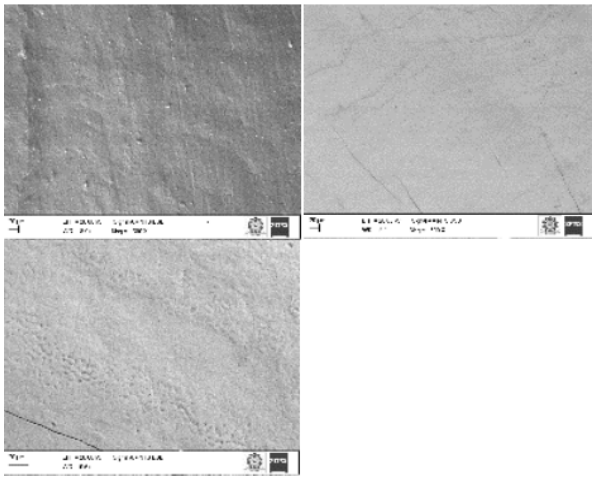
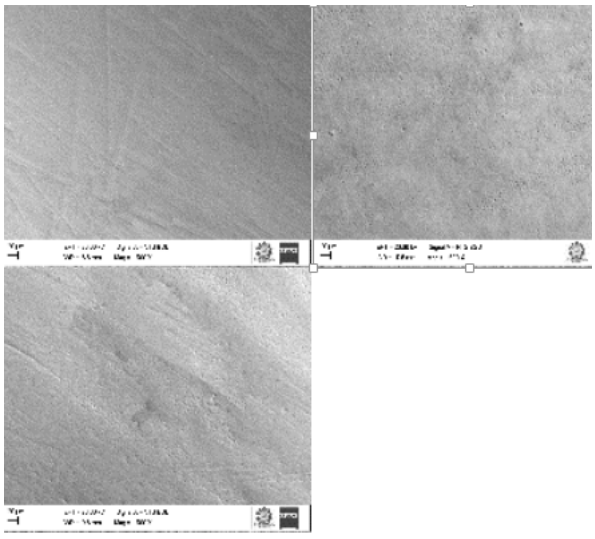
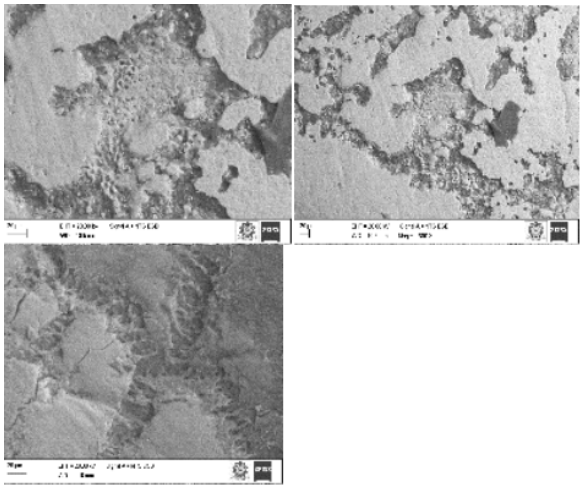


REMINPRO

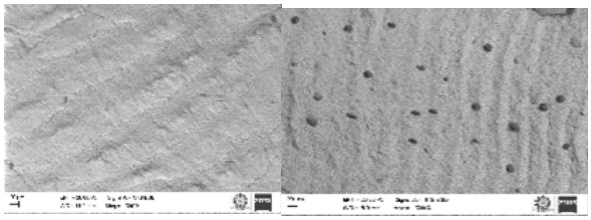


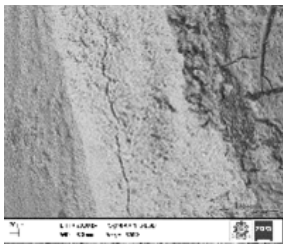
FINAL ACID CHALLENGE

ARTIFICIAL SALIVA

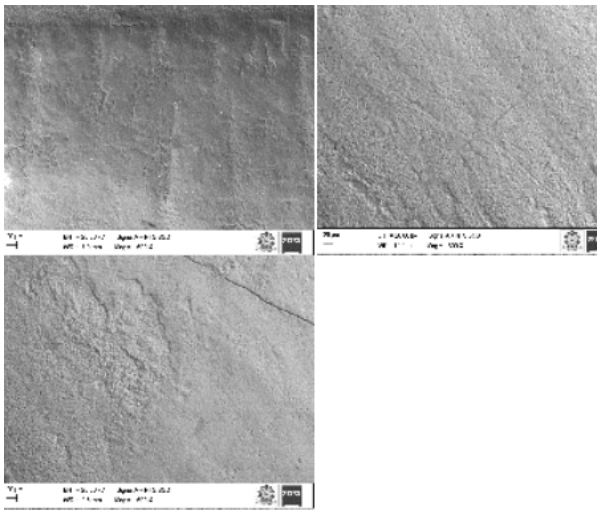


TOOTH MOUSSE PLUS

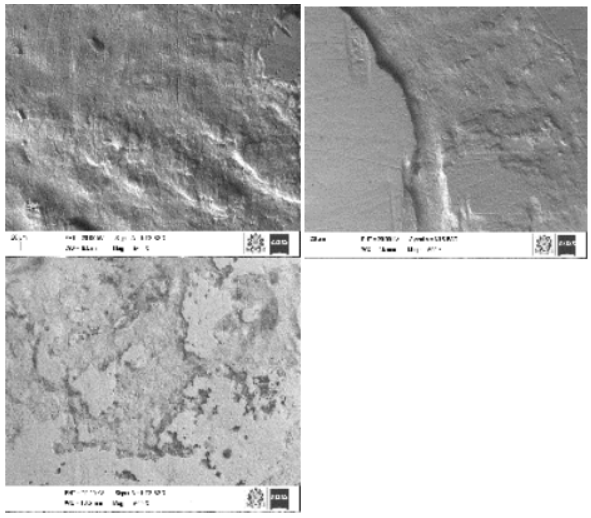




ENAFIX



REMINPRO



DISCUSSION

Despite of different forms of calcium and phosphorous compounds incorporated in all these products, all remineralizing agents examined showed higher remineralizing potential compared to their control as presented in SEM photos.

Natural tooth substance consists largely of hydroxyapatite that chiefly consists up of calcium and phosphate ions that is is being mimicked in the commercially available product REMIN PRO (VOCO) that tends to fill the superficial enamel lesions. [7]

A milk protein namely Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP) was so been introduced as a remineralizing agent in the year of 1998. This technology was developed by Eric Reynolds and co-workers at Melbourne, a formulation with incorporated fluoride to a level of 900 ppm (GC TOOTHMOUSSE PLUS ™). [8]

And the newer mode of remineralization without fluoride which contained, calcium sucrose phosphate (CaSP) was introduced as

ENAFIX (GROUP PHARMACEUTICALS) in the Indian market as a remineralizing agent. This product usually decomposes to calcium, phosphate, and sucrose ions, thus resulting in increased rate of remineralization. [9]

During the process of enamel remineralization the crystal voids repair and the repaired crystalline phase formed will depend on the concentration of bioavailable fluoride, calcium and phosphate ions. However several studies reported that the bioavailability of active fluoride ions is the critical factor for promoting remineralization and inhibition of demineralization.[12]

Reynolds et al in their study showed CPP-stabilized calcium phosphate solutions were shown to remineralize subsurface lesions. Although most of the remineralizing solutions were supersaturated with respective amorphous and crystalline calcium phosphate phases and concluded that the remineralizing capacity was greater for the solutions with the higher levels of CPP-stabilized free calcium and phosphate ions [13]

Sharma et al 14 evaluated the remineralization potential of CPP–ACP and HAp on the demineralized enamel surface and concluded that ReminPro yielded better results with regard to microhardness than GC Tooth Mousse. CPP-ACP cream used in their study does not contain fluoride unlike our study where we compared the remineralizing efficacy of CPP – ACPF (superior to the remininralizing efficacy of HAp (ReminPro) with 1,450ppm of fluoride. [14]

Jayrajan et al evaluated the efficacy of CPP-ACP and CPP-ACPF on the surface enamel remineralization using SEM and DIAGNOdent and they concluded that CPP-ACPF could be included in the routine hygiene and maintenance for reversing or arresting white spot lesions.[15]

Our study was in accordance to the study done by Gade et al who compared the remineralizing efficacy of Enafix with ooth Mousse Plus and suggested that that Enafix being a cost effective material as compared to GC Tooth mousse plus can be used as an alternative regime for remineralization.[16]

Another study by Kakkar et al that lies in accordance to our study where they found that GC Tooth Mousse Plus group was found the best among different medicaments, however it was not statistically significant, while Enafix gave better results. During brushing, calcium sucrose phosphate in Enafix quickly breaks down and releases calcium, phosphate, and sucrose phosphate ions into saliva. [17]

Another study by Tahmasbi et al whose results are not significant with our results, who evaluated the remineralizing efficacy of Remin Pro, CPP-ACP-F and NaF and they concluded that MI Paste Plus and to a lesser degree 0.05% NaF are effective for treatment of WSLs. Although Remin Pro had an efficacy superior to that of artificial saliva, its remineralizing effect seems to be inconsistent. [18]

CONCLUSION

1. Caesin phosphopeptide amorphous calcium phosphate containing 900ppm of fluoride has better remininerali zation when compared to other groups.
2. Calcium Sucrose Phosphate has the highest resisitant to acid attack when compared to other groups.
3. Enafix being a cost effective material when compared to GC Tooth Mousse Plus, can be used as an alternative for better remineralization.

Within the limitations of this particular in-vitro study, the above conclusions are so being derived.

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