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Dentistry

COMPARATIVE EVALUATION OF REMINERALIZING POTENTIAL OF DIFFERENT COMMERCIALLY AVAILABLE REMINERALIZING AGENTS : AN IN VITRO SCANNING ELECTRON MICROSCOPY/EDS STUDY

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ABSTRACT **Background -** Modern dentistry aims at non-invasive & biological approach using remineralizing agents for management of early carious lesion. Aim- To evaluate and compare the remineralizing potential of three commercially available remineralizing agents containing SDF(Fagamin), CPP-ACPF(GC Tooth Mousse Plus) and BiominF(Elsenz) on demineralized enamel. **Material and methods-** Samples from forty extracted premolar teeth were selected and subjected to demineralizing solution for 96 hrs to create white spot lesion. The demineralized samples were randomly allocated into four groups:Group I(control group), Group II(SDF),Group III(CPP-ACPF),Group IV(Elsenz). White spot lesion containing demineralized samples were subjected to the test agents for 5 minutes everyday for 30 days. Scanning electron microscope (SEM) and Energy dispersive Spectroscopy (EDS) analysis were carried out at pre-remineralization and post-remineralization stage.**Statistical Analysis-** The data were analyzed using SPSS 19.0 software with one-way ANOVA and paired t-test. **Results-** All three test agents showed significant remineralization of previously demineralized lesions. However, Elsenz showed the greatest remineralization, followed by SDF and then CPP-ACPF paste. **Conclusion-** Elsenz proved to be the best remineralizing agent and can be strongly recommended as an alternative to other remineralizing agents used in this study.

KEYWORDS : Remineralizing potential, Early carious lesion, SEM-EDS

INTRODUCTION:

Dental caries is a highly prevalent disease worldwide. It is initiated through demineralization of tooth hard tissue. Demineralization and Remineralization are balanced processes occurring naturally in the oral cavity1 where mineral exchange occurs naturally at surface of tooth and saliva, but if natural balance gets hampered,pH drops and dental caries occurs.

Many studies have shown that early carious lesions have a potential for remineralization, particularly with the use of enhanced remineralizing agents.

This study aimed to comparatively evaluate remineralizing potential of SDF,CPP-ACPF and Elsenz as literature is scanty on comparison of remineralization potential of these three remineralizing agents altogether.

MATERIAL AND METHODS:

The present in vitro study was carried out in the Department of Pediatric and Preventive Dentistry, D.J. College of Dental Sciences & Research, Modinagar in collaboration with Institute Instrumentation Centre, Institute of Technology – Roorkee.

Total forty human premolar teeth extracted for orthodontic reasons fulfilling the inclusion criteria were used for the study within three months of extraction as per recommendations of occupational safety and health administration guidelines (OSHA).

Teeth with intact crown structure, without any visible caries, free from restoration and fluorosis ,without any stains or cracks, without any hypoplastic lesion or white spots on any surface of teeth were included in the study. Hypoplastic or hypomineralised tooth, presence of white spot lesions and crown of the tooth fractured during extraction were excluded from the study.

Methodology

1.Preparation of samples:-

Forty permanent non-carious premolars were selected, cleaned of soft tissue and debris and stored in 10% formalin solution. To limit the area of study, a white stick – on paper of 2mm x 4 mm dimension on the buccal surface in the middle 1/3rd of the crown was stuck on each tooth sample and then acid – resistant nail varnish was applied on the rest of the surface. Later, stick-on paper was removed to expose the area of the buccal surface that needs to be treated. Demineralizing solution was prepared in the the department of biochemistry. The composition of the demineralizing solution was 2.2 g calcium chloride (CaCl2.2H2O), 2.2 g potassium hydrogen orthophosphate, 2.85 mL acetic acid, 56 g potassium hydroxide,1050 ml distilled water. Ph was adjusted to 4.5. All specimens were immersed in the demineralizing solution for 96 hrs until white spot lesions appeared on the samples. Samples were then sectioned to obtain a derooted buccal half of the crown structure.

2. Scanning electron Microscope analysis pre- remineralization:-

Demineralized samples were air dried, gold sputtered and were subjected to SEM/EDS for the analysis of demineralization.

3. Division of Samples:-

All the 40 demineralized samples were randomly allocated into four groups as follows:-Group I – control group, Group II- SDF 38%, Group III- CPP-ACPF, Group IV- BiominF;Elsenz

4. Application of Remineralizing agents:-

Group I (control group) was subjected only to natural saliva having the neutral pH for 24 hrs at 37 degree Celsius to simulate oral environment and not to any remineralizing agent. Group II was treated with SDF 38%, Group III was treated with CPP-ACPF and Group IV was treated with BiominF;Elsenz with the help of an applicator tip. The samples treated were rinsed in normal saline, and kept in natural saliva. Before the application of remineralizing agent on every other day, the samples were air dried with the help of three-way syringe and saliva was replaced with freshly collected natural saliva. The procedure was repeated for 5 minutes everyday for 1 month.

5. Scanning electron microscope analysis post remineralization:-
All the samples treated with remineralizing agents were again analyzed by SEM/EDS analysis.

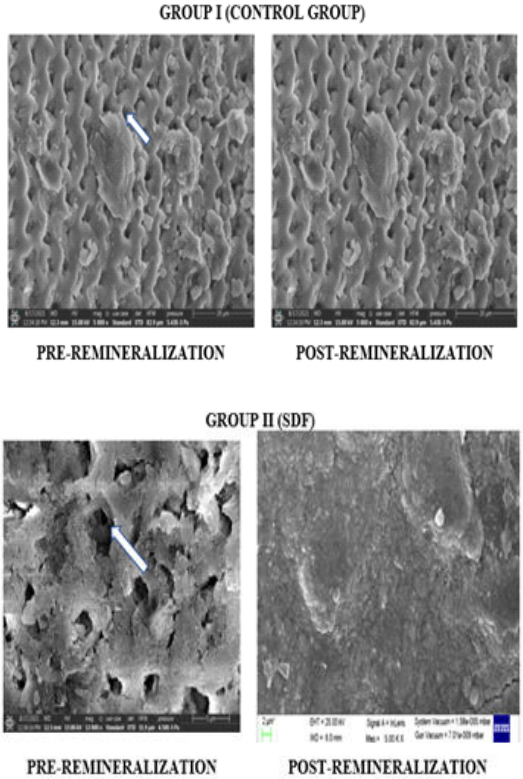
RESULTS

SEM images revealed that there is microporosities seen in pre-remineralization images for all groups evident of demineralized areas wheras less microporosities evident of remineralized are seen in post remineralization images in all three groups other than Group I, showing minimal to no change. The demineralized areas has been indicated for all groups with arrows in Figure 1.

Mean change of calcium weight %, phosphate weight% and changes in SEM images grading was highest in Group IV followed by Group II, Group III, Group I respectively. Intragroup comparison by paired t-test showed that all the test groups except Group I was significant (Table 1). On one-way Anova analysis, change in calcium weight percentage, change in phosphate weight percentage and change in SEM images grading showed significant results($P<0.05$)(Table 2).

SEM images were statistically analysed on the basis of Bonetti et al grading criteria2 as follows:-

Grade	Status
0	Enamel surface remained perfectly intact with no grooves, pits, and porosity
1	Presence of surface irregularities on enamel surface, without demineralization of prismatic and/or interprismatic enamel
2	Presence of wrinkles and demineralization of prismatic/interprismatic enamel
3	Diffuse demineralization involved the rod core, with decomposition of morphology of prism



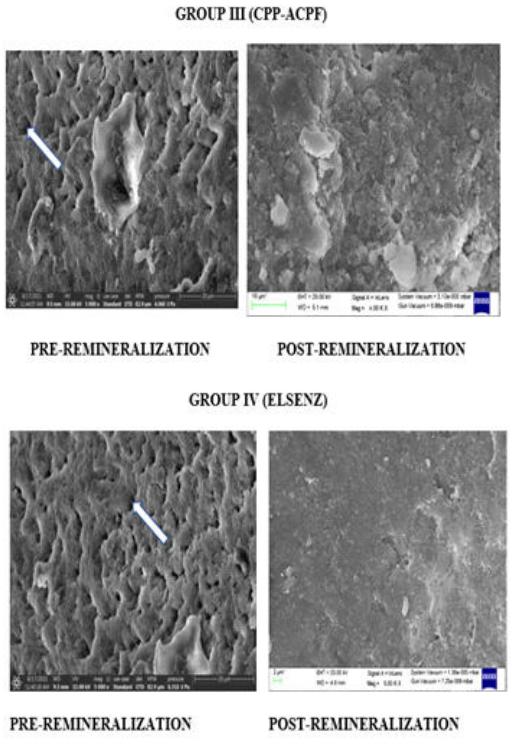


Figure 1. SEM surface morphology change in all groups.

Table 1. Intragroup comparison of calcium and phosphorous weight percentage

Groups	Calcium weight % (Mean Change)	t value for calcium weight % (Mean Change)	P value for calcium weight % (Mean Change)	Phospho-rous weight % Mean Change	t value for phospho-rous weight% (Mean Change)	P value for phospho rous weight% (Mean Change)	Mean Change of SEM images grading	t value for mean Change of SEM images grading	P value for mean Change of SEM images grading
Group I	0.00±0.00	0.000	1.000	0.00±0.00	1.000	1.000	0.00±0.00	0.000	1.000
Group II	1.00±0.98	2.428	0.011*	0.64±0.96	0.929	0.047*	1.80±0.63	9.000	0.001*
Group III	0.52±0.37	1.765	0.001*	0.32±0.37	0.976	0.001*	1.50±0.52	8.123	0.001*
Group IV	1.50±1.37	2.891	0.001*	1.25±1.37	1.569	0.01*	2.20±0.63	10.113	0.01*

*P value ≤ 0.05 (significant)

Table 2. ONE-WAY ANOVA test for Comparison of means of changes in calcium and phosphorous weight% and SEM images grading among various groups

ONE-WAY ANOVA	Mean Square for calcium weight% change	F value for calcium weight% Mean Change	P value for calcium weight % Mean Change)	Mean Square for phosphate weight% change	F value for phosphate weight %	P Value for phosphate weight %	Mean Square of SEM images grading	F value for mean Change of SEM images grading	P value for mean Change of SEM images grading
Between Groups	4.084	5.423	.003*	49.270	7.211	0.001*	9.225	34.237	0.001*
Within Groups	.753			40.702			0.269		
Total									

*P value ≤ 0.05 (significant)

DISCUSSION

White spot lesion can be the earliest sign of tooth decay and can be reversible only if detected and treated at an initial stage. Numerous remineralizing agents have been used for remineralization as it does direct delivery of ions to the affected area[3]. In recent times, to better suit the dynamic nature of reversibility of initial carious lesion, application of commercially available remineralizing agents has been a convenient non-invasive & biological approach rather than the conventional surgical approach. In the present invitro study, three

remineralizing agents namely SDF, CPP-ACPF and Elsenz has been compared.

CPP-ACPF has an additional beneficial effects of fluoride along with calcium and phosphate at the enamel surface. It helps in the formation of fluoroapatite crystals that are more resistant to dissolution by acids and enhances the process of remineralization[4]. Silver Diamine Fluoride is a topical fluoride solution with a concentration of 38% that inhibits the cariogenic biofilm production[5]. Fluoride in SDF causes an increase in calcium and phosphate ions due to formation of calcium fluoride and silver phosphate[6].Elsenz is an effective remineralizing agent containing fluoride (530ppm) with bioactive glass(BiominF) that form nanocomplexes at enamel surface. It causes a localized transient increase in Ph and cause precipitation of calcium and phosphate on the tooth surface[7].

In the present study, SEM/morphological analysis of enamel surface as well as EDS for mineral content evaluation i.e change in calcium weight percentage and phosphate weight percentage was done. SEM images analysis showing changes in surface morphology of enamel was done and scored according to Bonetti et al grading criteria[2].

According to microscopic results obtained from the images of SEM, the pre- remineralization samples showed deprived interprismatic and prismatic substances of enamel in all groups. And post-remineralization, Group IV treated with Elsenz showed maximum remineralized enamel with uniform ion deposition. Similiar study was done by Sivaranjani S et al 2018[8] showed that that the SEM pictures revealed the maximum remineralization in Elsenz. Group II treated with SDF showed less areas of remineralization than Elsenz but more than CPP-ACPF in accordance to study done by Yadav RK et al 2022[9],Vinod D et al 2020[10]. Group III treated with CPP-ACPF showed less areas remineralization in comparison to Group IV followed by Group II but was more than that of Group I in accordance to study by Reynold et al 1997[11],El Hagry BH et al 2021[12].

The non significant change in Group I was because samples were subjected only to saliva and not to any remineralizing agent, saliva although having some remineralization potential couldn't raise the amount of calcium and phosphate excretion by itself, in absence of remineralizing agents. This is in accordance to study done by Kamal D 2018 et al[13].

On EDS analysis, mean change of Group IV; Elsenz was more than SDF and CPP-ACPF. This is because of its active ingredient fluoro calcium phosphosilicate,a newer generation of bioactive glass[12].It causes sustained and prolonged release of Ca+ and P− ions that increases the concentration of available calcium and phosphate required for the remineralization process of initial carious lesion[14]. The particles of Elsenz attach to the tooth surface and continue to release ions and re-mineralize the tooth surface after its initial application. The bioactive glass have been shown, in-vitro studies to release ions and transform into hydroxyapetite for up to 2 weeks[15]. During acid attack at lower pH, bioactive glass particles dissolves rapidly in saliva, and release calcium, phosphate and fluoride ions so that neutralization effect takes more rapidly[16]. It has caused Elsenz to precipitate more amount of calcium and phosphate rapidly in comparison to that of SDF and CPP-ACPF. Bioactive glass releases hydroxycarbonate apatite (HCA) directly, without the intermediate ACP phase[15] that occurs in case of CPP-ACPF making it to take more time to act in comparison to Elsenz. Thus, rapid dissolving in saliva and prolonged and sustained release of Ca+ and P− ions caused Elsenz to show the most significant remineralizing potential. Study done by Srivastava S. 2019 et al.[17] showed that Elsenz proved to have better remineralizing potential than GC Tooth Mousse Plus and Sensodyne Repair which is in accordance to the present study. Other studies supporting the remineralizing efficacy of BiominF;Elsenz are by Sivaranjani S 2018 et al.[8] and N Nagaveni 2022 et al[18].

On EDS analysis, mean change of SDF being more than CPP-ACPF is attributed to the flowability of the SDF solution. The liquidity of SDF allowed for the full contact of SDF and the enamel surface[19] in a relatively shorter time as compared to CPP-ACPF. Thus, SDF showed more remineralizing potential than CPP-ACPF but less than Elsenz. Study done by Yadav RK et al 2022[9] showed that SDF resulted in same remineralizing potential under scanning electron microscopy and energy dispersive X-ray analysis as in the present study, followed by CPP-ACFP, CPP-ACP, Fluoride toothpaste, and Fluoride-free toothpaste. On EDS analysis, Mean change of CPP-ACPF was least i.e. although it showed remineralizing potential but was least effective than the other experimental groups. According to Mehta et al. 2014[20] it is suggested that CPP-ACP molecules need an acidic exposure to get activated and separate ACP from the casein. But when it was necessary for activation they got washed away by saliva[21]. And according to Thierens L AM 2018 et al.[22] long term application of CPP-ACPF needs time to take effect.

Thus, the results obtained in the study indicate that each of the remineralizing agents showed remineralizing potential however BiominF;Elsenz proved to be the most efficient of all in reverting back the demineralized enamel close to as that of sound enamel. And moreover Elsenz is the most cost effective than SDF and CPP-ACPF. And it is also proved to be esthetically pleasant as it doesn't cause blackish discolouration on application as in the case of SDF. Thus, Elsenz can be recommended as an effective remineralizing agent.

CONCLUSION

Elsenz(BiominF) proved to be the best remineralizing agent in terms of increased Calcium and Phosphorous weight percentage under EDS and improved surface morphology of enamel under SEM. Thus, Elsenz can be strongly recommended as an efficient remineralizing agent.

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