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Journal of Contemporary Orthodontics

Journal homepage: https://www.jco-ios.org/

Original Research Article

Evaluation of efficacy of various remineralizing agents on artificially demineralized human enamel – An in-vitro study

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ARTICLE INFO

Article history:
Received 27-09-2022
Accepted 09-01-2022
Available online 29-03-2023

Keywords:
White spot lesions
Remineralization
Laser fluorescence
SEM.

ABSTRACT

Introduction: Currently much research revolves around remineralization as incidence of white spot lesions in patient undergoing orthodontic treatment is 2-96%. The objective of this study was to find out the efficacy of Casein phosphopeptide-amorphous calcium fluoride phosphate (CPP-ACFP), Functionalized tricalcium phosphate (f-TCP), Calcium sodium phosphosilicate (CSPS), Calcium sucrose phosphate (CSP), Nano hydroxyapatite (nHAP) and Fluoride (F) in remineralizing artificially demineralized enamel and DIAGNOdent and scanning electron microscope were used to record the changes.

Materials and Methods: The study included 140 extracted premolars which were divided into 7 groups in which 6 were experimental groups: I (CPP-ACPF), II (f-TCP), III (CSPS), IV (CSP), V (nHAP), VI (F) and 1 control group: VII (C). All the samples were evaluated using DIAGNOdent at the baseline, post demineralization and post remineralization. One sample was randomly selected from each group for evaluation using SEM.

Results: Statistical analysis has shown that all the groups had higher amount of remineralization except control group. CSPS showed maximum remineralization.

Conclusion: All the artificially demineralized samples treated with remineralizing agents for 3 weeks reduced the severity of lesions by forming calcific deposits. However CSPS showed marginally more amount of remineralization than others.

1. Introduction

White spot lesions are considered as undesirable and unaesthetic consequences of fixed orthodontic appliance therapy. White spot lesions are defined as the, "subsurface enamel porosity from carious demineralization which can be seen as milky opacity when located on smooth surface".[1] Dental caries is disease process where equilibrium exists between demineralization and protective factors which causes remineralization[2] which lead to the idea of remineralizing the White Spot Lesions. Orthodontic appliances makes maintaining oral hygiene difficult causing more amount of plaque accumulation which further results in demineralization around brackets.

Presence of fixed orthodontic appliances causes deposition of fermentable carbohydrates and the adhesion of S. mutans. As a result, demineralized white spot lesions often become an unavoidable consequence of orthodontic treatment. WSL can be clinically visible as early as 1 month after fixed appliance is bonded. It is estimated that 50% of patients develop WSL in at least one tooth by the end of orthodontic treatment. The prevalence of white spot lesions

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https://doi.org/10.18231/j.jco.2023.004

among orthodontic patients treated with fixed appliances varies from 13% to 75% and can go as high as 97%.[3] Children within age groups of 11–14 years are at more risk of developing these lesions.[4] The white spot lesions mainly occur in the maxillary anterior dental region which gives unaesthetic appearance at the end of orthodontic treatment which is undesirable.

Risk of decalcification and WSL formation can be minimized by following proper diet including low carbohydrates and by maintaining adequate oral hygiene. Remineralization is the natural process to halt the process of demineralization of enamel which stop or reverse the progressing lesion and is based on calcium, phosphate ions with the help of fluoride to rebuild a new surface on existing crystal remnants in subsurface lesions remaining after demineralization.[5–9]

This In-Vitro study was undertaken to compare the efficacy of remineralizing agents containing Casein phosphopeptide-amorphous calcium fluoride phosphate (CPP-ACFP), Functionalized tri-calcium phosphate (f-TCP), Calcium sodium phosphosilicate (CSPS), Calcium sucrose phosphate (CSP), Nano hydroxyapatite (nHAP) and Fluoride (F) on remineralization of WSL using DIAGNO dent instrument (KaVo, Germany) and SEM.

2. Materials and Methods

The study followed an In-Vitro experimental design where the sample included one hundred and forty extracted premolar teeth collected from the Department of Oral and Maxillofacial Surgery, from orthodontic patients undergoing therapeutic extraction of premolars.

2.1. Sample preparation

142 premolars which were extracted for orthodontic procedures were collected and cleaned under running water with a tooth brush. All the samples were randomly divided into seven groups containing 20 teeth in each group using simple randomized sampling. Each sample tooth was painted with an acid resistant varnish leaving a window of 3 × 3mm on buccal surface; this was done to limit the area of study. Seven different colored varnishes were used for ease of identification.

2.2. Recording of baseline value with diagno dent and sem analysis

All the samples were analyzed with the help of DIAGNOdent® (KaVo, Biberach, Germany) to assess surface changes present on the labial window (Figure 2). Type B probe is used in this study in order to collect the fluorescence from all directions on tooth surface. Samples showing a moment value between 3 and 7 on the digital display were selected. The baseline values of the six groups were then recorded and one sample was subjected to baseline SEM evaluation, at 1000 x magnification at 10 Kv to analyze the surface topography.

2.3. Preparation of demineralizing solution

The demineralization solution was prepared in the Department of Biochemistry, Panineeya Institute of Dental Sciences and Research Center. 1000 ml of demineralizing solution was prepared (Figure 3) by mixing 2.2 mM KH2PO4, 0.05M acetic acid, 2.2 mM CaCl2 and pH adjusted to 4.4 by adding 1 M KOH to the prepared solution.[10]

2.4. Demineralization procedure

Each sample was immersed individually into separate containers which were numbered from 1 to 20 in each group, each containing 4 ml of demineralizing solution, for a period of 96 hours (Figure 4). This demineralizing procedure produced a consistent subsurface lesion in all enamel samples. After demineralization the samples were taken out, washed with distilled water, dried, and placed back in their respective containers. The teeth were evaluated with DIAGNOdent® and the samples showing a moment value of 9 and above were taken for further evaluation. The readings were recorded for statistical analysis. One sample was subjected to SEM for assessing the surface topographic changes after demineralization.

2.5. Remineralizing procedure

The remineralizing solution was prepared by using 1.5 mM CaCl2, 0.9 mM NaH2PO4, and 0.15 M KCl and had a pH of 7.[11–16] The samples in each group were treated with the respective remineralizing agent for 21 days twice daily, rinsed with distilled water, dried, and stored in the remineralizing solution.

2.6. Remineralizing agents

1. Group I: Casein phosphopeptide - Amorphous Calcium Fluoride Phosphate (CPP-ACFP)
2. Group II: Functionalized Tricalcium phosphate (f-TCP)
3. Group III: Calcium sodium phosphosilicate (CSPS)
4. Group IV: Calcium sucrose phosphate (CSP)
5. Group V: Nano-hydroxyapatite (nHAP)
6. Group VI: Fluoride (F) Group VII: Control (C)

All the test groups were applied their respective remineralizing pastes twice daily on exposed enamel window of all the samples with applicator tip and let undisturbed for 3 minutes and then rinsed with distilled water, dried and stored in individual containers containing remineralizing solution. Nothing was applied in control group.

All the samples were dried at day 21 and the surface was assessed using DIAGNO dent to record the values after the remineralization procedure has been completed. To assess the remineralized surface changes on the enamel, one sample from each group was randomly selected and compared with each other using SEM.

3. Statistical Analysis

Normality of data was tested using Kolmogorov Smirnov test. Non-parametric tests like Kruskal Wallis ANOVA for inter group comparison along with Mann-Whitney U test for multiple group comparisons and Wilcoxon matched pairs test for intra group change was used. It was seen that data was not normally distributed Hence Non-parametric Tests like Kruskal-Wallis ANOVA for inter group comparison (Table 1) along with Mann-Whitney U test for multiple group comparisons and Wilcoxon matched pair test for intra group change was used.

Figure 1: Recording baseline score by DIAGNO dent

4. Results

The mean values at Baseline were highest for Group II (f-TCP) and Group V (nHAP): 3.85 ± 0.37 followed by Group I (CPP ACFP): 3.80 ± 0.41, Group VI (F): 3.75± 0.44, Group IV (CSP) and Group VII (C): 3.65 ± 0.49, Group III (CSPS): 3.45 ± 0.51 with no significant difference (0.0610). Post demineralization values increased in all groups and was highest for Group I (CPP ACFP) and Group IV (CSP): 9.60±0.60, followed by Group III (CSPS), Group VI (F), and Group VII (C) which had similar score 9.55±0.51, Group II (f-TCP): 9.50±0.51, Group V (nHAP): 9.10±0.31 and showed significant difference (0.0360).

Also the change from post demineralization to post remineralization was significant among all groups (0.0001). 21 days post remineralization mean value was highest for Group VII (C) 8.50±0.61 followed by Group VI (F) 7.20±0.41, Group 2 (f-TCP) and Group IV (CSP) 6.80±0.41 had similar score, Group I (CPP ACFP) 5.80±0.41, then Group V (nHAP) and Group III (CSPS) 5.70±0.47 both

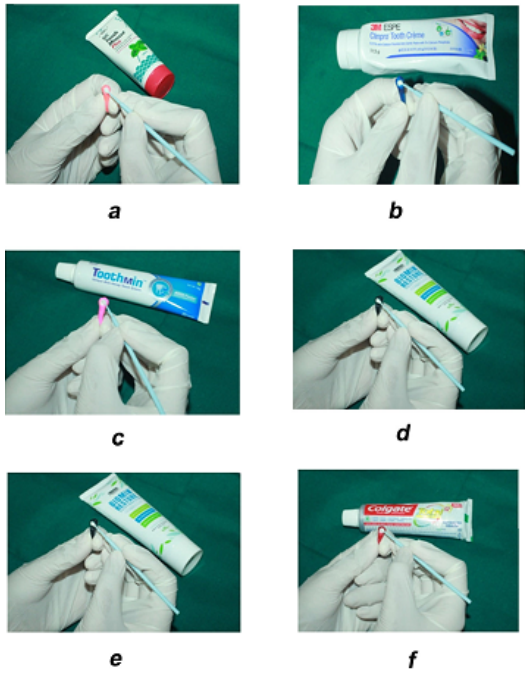

Figure 2: Application of remineralizing agents

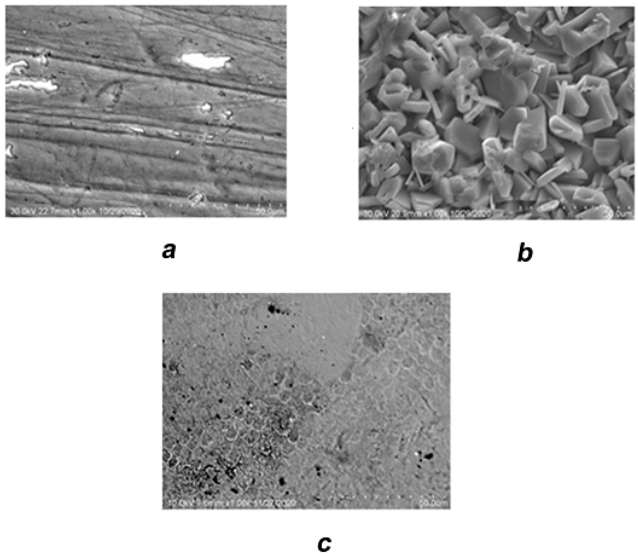

Figure 3: a: SEM image of normal enamel surface **b:** SEM image of demineralized enamel surface **c:** SEM image of post remineralizzed control group

Table 1: Comparison of seven groups with Baseline, post demineralization and post remineralization scores by Kruskal Wallis ANOVA

Groups	Baseline					Post demineralization					Post remineralization				
	Mean	SD	Median	IQR	Mean Rank	Mean	SD	Median	IQR	Mean Rank	Mean	SD	Median	IQR	Mean Rank
CPP-ACFP	3.80	0.41	4.00	0.00	76.50	9.60	0.60	10.00	0.50	76.63	5.80	0.41	6.00	0.00	35.70
f-TCP	3.85	0.37	4.00	0.00	80.00	9.50	0.51	9.50	0.50	71.50	6.80	0.41	7.00	0.00	82.50
CSPS	3.45	0.51	3.00	0.50	52.00	9.55	0.51	10.00	0.50	74.95	5.70	0.47	6.00	0.50	32.30
CSP	3.65	0.49	4.00	0.50	66.00	9.60	0.60	10.00	0.50	76.63	6.80	0.41	7.00	0.00	82.50
nHAP	3.85	0.37	4.00	0.00	80.00	9.10	0.31	9.00	0.00	43.90	5.70	0.47	6.00	0.50	32.30
F	3.75	0.44	4.00	0.38	73.00	9.55	0.51	10.00	0.50	74.95	7.20	0.41	7.00	0.00	98.80
C	3.65	0.49	4.00	0.50	66.00	9.55	0.51	10.00	0.50	74.95	8.50	0.61	8.00	0.50	129.4
H-value			12.0240					13.5180			116.6360				
P-value			0.0610					0.0360*			0.0001*				

*p<0.05

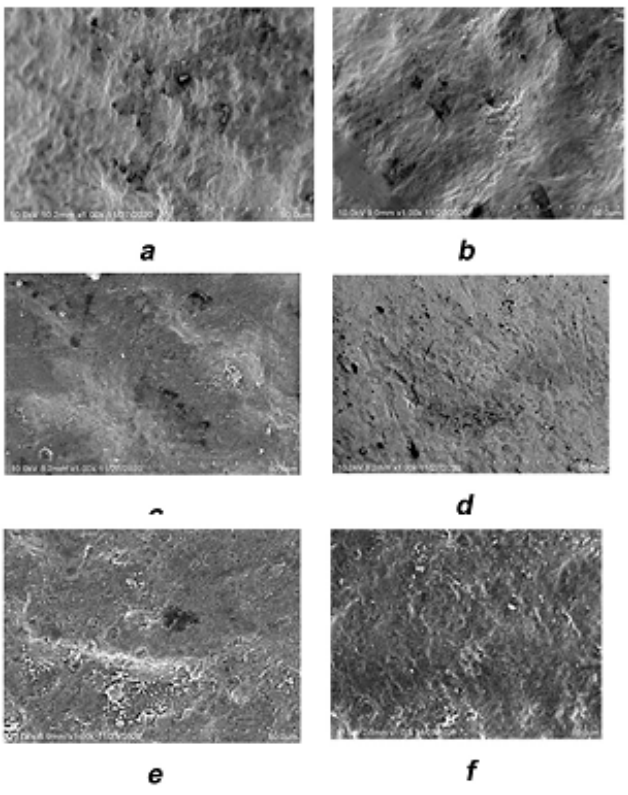

Figure 4: a: SEM image of post remineralized CPP-ACFP group **b:** SEM image of post remineralized CSPS group **c:** SEM image of post remineralized nHAP group **d:** SEM image of post remineralized f-TCP group **e:** SEM image of post remineralized CSP group **f:** SEM image of post remineralized fluoride group

had least mean value. The lowest mean value indicates the maximum remineralization.

The mean value in Group I (CPP-ACFP) at baseline is 3.80±0.41 which increased to a mean value of 9.60±0.60 post demineralization and decreased to 5.80±0.41 post remineralization. Thus, there was a mean difference of 3.80±0.62 showing significance (p = 0.0001). Group II (f-TCP) had a mean baseline value of 3.85±0.37 which increased to a mean value 9.50±0.51 post demineralization and decreased to 6.80±0.41 post remineralization. The mean difference was 2.70± 0.73 which was significant (p =0.0001). Group III (CSPS) had a mean baseline value of 3.45±0.51 which increased to a mean value 9.55±0.51 post demineralization and decreased to 5.70±0.47 post remineralization. The mean difference was 3.85±0.75 which was significant (p =0.0001). Group IV (CSP) had a mean baseline value of 3.65±0.49 which increased to a mean value of 9.60±0.60 post demineralization and decreased to 6.80± 0.41 post remineralization. The mean difference was 2.80±0.77 which was significant (p =0.0001). Group V (nHAP) had a mean baseline value of 3.85±0.37 which increased to a mean value of 9.10±0.31 post demineralization and decreased to 5.70± 0.47 post remineralization.

SEM was done to investigate the morphology and surface changes at baseline (normal sound enamel), post demineralization and post remineralization. Figure 3 a shows the SEM images of the normal enamel surfaces showing uniformly smooth surface. Figure 3 b shows demineralized enamel appearing as depressions and irregularities resulted from destruction of enamel surface when placed in demineralizing solution. The samples (Control group) which were placed in remineralizing solution without applying any remineralization agents showed negligible amount of remineralization showing faint lines of mineralization around the porosities (Figure 3 c).

All samples showed significant amount of remineralization of artificially demineralized enamel. Demineralized enamel treated with CPP-ACFP showing relatively smooth enamel surface with very few concavities (Figure 4 a). Demineralized enamel treated with Calcium sodium phosphosilicate (Bioactive glass) shows dense calcified deposits displaying thick and more frequent lines of remineralization (Figure 4 b). SEM image of demineralized enamel sample treated with nano-HAP and functionalized Tricalcium phosphate shows normal enamel surface; smooth calcific deposits covering the area of demineralization (Figure 4 c & d). Samples treated with Calcium sucrose phosphate and Fluoride reveals only few deposits which are profusely scattered along the porous defects (Figure 4 e & f).

5. Discussion

The White spot lesions (WSL) are defined as "subsurface enamel porosity from carious demineralization" that presents itself as "a milky white opacity, when located on smooth surfaces".[11,12] The scope for remineralizing incipient carious lesion came into light due to concept of remineralzing and demineralizing cycle. In present study, 6 different remineralizing agents were chosen to find the efficacy of their remineralizing potential.

In group I (CPP-ACFP), GC Tooth Mousse Plus was used which contains additional 0.2% or 900 ppm of sodium fluoride along with CPP-ACP which comprises nanocomplexes of milk protein. It has more amounts of localized free calcium phosphate and fluoride ions, which helps in reducing demineralization.[13–16]

In group II (f-TCP), Clinpro Tooth Crème was used which contains 950 ppm sodium F with 500 ppm functionalized tri-calcium phosphate, where the tri-calcium phosphate particles have been ball milled with sodium lauryl sulphate to produce particles within the size range of 1 μm to 15 μm2 and organic calcium phosphate hybrid is formed which protects calcium oxides are from interacting with fluoride, preventing calcium phosphate reaction with fluoride and formation of calcium fluoride. This makes calcium, fluoride and phosphate available in an aqueous form which enables remineralization process.[17]

In group III (CSPS), Biomin Restore tooth paste was used which contains Calcium sodium phosphosilicate (Bioactive glass). It is a biocompatible material which was developed as bone regenerative material initially. It consists of calcium, phosphorus, silica and sodium which enhance remineralization. It continuously deposits crystalline hydroxyl-carbonate apatite (HCA) which has a structure which resembles human tooth mineral. When bioactive glass contacts saliva, sodium ions are released which increases the pH which is suitable for Hydroxyapatite formation.[2]

In group IV (CSP), Toothmin toothpaste was used which contains Calcium Sucrose phosphate commonly known as "anticay." It provides freely available calcium and phosphate ions at concentrations significantly higher than those normally present in the saliva at pH values found in the oral cavity.[18–21] CSP acts as a cariostatic agent through different mechanisms and prevents development of WSLs.

In group V (nHAP), Aclaim toothpaste was used which contains nHAP. HA (Ca10 (PO4)6(OH)2) is a major component of the hard tissue in the human body. Currently HA toothpaste has gained wide attention for tooth whitening and remineralization.[22,23] Tooth has its bright white appearance due to hydroxyappetite as its major component.

In group VI (F), Colgate Total toothpaste was used in which the active ingredient is 1450 ppm sodium fluoride. Fluoride has strong cariostatic property which helps in preventing dental caries. The widespread use of toothpastes containing fluorides has declined dental caries among various countries.[24–26]

All the six remineralizing agents mentioned above, having different composition and mechanism of action were potentially able to remineralize the enamel.[27–29]

SEM analysis at 1000 magnification revealed that the normal enamel shows smooth and intact surface with fish scale appearance. Post demineralization samples showed histological features similar to dental caries and have been successfully used to study the demineralization of enamel in-vitro as discussed by Soumya et al. in 2011.[30–32] The enamel surface showed increased porosities and had uneven and rough surface and a honeycomb pattern of demineralized enamel was observed.

All the test groups after remineralization was completed revealed a layer of homogenous surface deposition of minerals compared to the control group. The enamel rods and prismatic substance were not clearly well-defined, but calcified deposits containing irregular crystals were evident. The enamel topography after 21 days of remineralization of Group I and III (CPP-ACFP and CSPS) showed areas of calcified deposits which were scattered in and around the porous defects. Enamel surface in these both groups had maximum remineralization when observed in SEM microphotographs. Scanning electron micrograph of demineralized enamel treated with nano-HAP (Group V) and functionalized Tricalcium phosphate (Group II) showed smooth calcific deposits covering all demineralized irregularities. Samples treated with Calcium sucrose phosphate (Group IV) and Fluoride (Group VI) reveals only few mineralized deposits which were scattered along the demineralized defects.

Group VII showed a very minimum amount of remineralization of enamel surface after completion of 21 days, which is in accordance with study conducted by Rirattanapong et al. Saliva is known to have some amount

of remineralization potential but does not increase the levels of calcium and phosphate release. After 3 weeks of remineralization, irregular surface with multiple porosities were still visible in the control group.

Drawbacks of the present study is period of remineralization which was only 21 days, which could not remineralize artificial caries completely, thus the exact period of application for complete remineralization cannot be described for remineralizing agents used in the study. Further study may be carried out to know if enamel could fully recover from the white spot lesions by using these remineralizing agents and the time it could take to complete the process. It is also recommended that the efficacy of these remineralizing pastes in In-Vivo conditions be investigated.

6. Conclusion

All the experimental groups showed significant remineralization of artificially induced lesions, but to varying degree. Among the 7 groups, maximum remineralization was seen with CSPS, followed by CPP-ACFP, nHAP, CSP, f-TCP, Fluoride and control. post reminerlization surface changes was found to be greatest with CSPS and CPP-ACFP as they formed thick calcific deposits masking demineralization when compared to CSP, f-TCP, nHAP, and fluoride. All the topically applied remineralizing agents used in this study help in remineralization of white spot lesions to great extent and can be recommended for the patients presenting with white spot lesions for efficient remineralization. Also these agents can be advised for prophylactic use from the commencement of orthodontic treatment to prevent white spot lesions.

7. Source of Funding

None.

8. Conflict of Interest

None.

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Cite this article: Rawal K, Manne RK, Gandikota CS, Shivaram N. Evaluation of efficacy of various remineralizing agents on artificially demineralized human enamel – An in-vitro study. *J Contemp Orthod* 2023;7(1):17-23.