EGYPTIAN
DENTAL JOURNAL

Vol. 64, 2757:2765, July, 2018

I.S.S.N 0070-9484

Fixed Prosthodontics, Dental materials, Conservative Dentistry and Endodontics

www.eda-egypt.org • Codex : 170/1807

THE EFFECTIVENESS OF A NANO-HYDROXYAPATITE PASTE AND A TRI-CALCIUM PHOSPHATE FLUORIDE VARNISH IN WHITE SPOT LESIONS REMINERALIZATION (RANDOMIZED CLINICAL TRIAL)

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ABSTRACT

Objective: To evaluate and compare clinically the effectiveness of a nano-hydroxyapatite (n-HAP) paste (Sesensibilize™ Nano-P, FGM) and a tri-calcium phosphate fluoride (TCP-F) varnish (Clinpro™ White Varnish, 3M ESPE) in remineralization of white spot lesions (WSLs) on young permanent teeth.

Methods: 20 patients having at least one pair of teeth with WSL were randomly selected to this study according to specific criteria. Each patient received two treatments of WSL; one treatment using n-HAP paste on one side of the arch (n-HAP group) and a second one using TCP-F varnish on the contralateral or opposing side of the arch (TCP-F group). A total of 80-teeth with WSLs were included in this randomized clinical trial. The reminaralizing agents were applied over the tooth surface with a WSL as per manufacturer recommendations. Four application sessions were completed during the first month on a weekly basis. DIAGNODENT PEN (DD) (Kavo Dental – Germany) was used to assess the degree of remineralization. DD readings were recorded at baseline, after one, three and six-months of the reminaralizing agents' application. All patients attended the treatment sessions and remained throughout the follow-up periods.

Results: There was a statistically significant difference between the WSLs mean DD readings over time for both groups (Friedman, $P<0.001$). The mean DD reading was 22.05 (±3.250) in n-HAP group and 21.33 (±3.467) in TCP-F group at the baseline, decreased to 10.80 (±1.7423) and 9.675 (±2.693) at one-month, 8.30 (±1.880) and 10.95(±2.726) at the 3-month, 7.875 (± 1.555) and 13.725 (±2.745) at the 6-month follow-up in n-HAP group and TCP-F group respectively. Upon comparing the groups, the WSLs had a similar mean DD reading at baseline ($P>0.05$). There was no statistically significant difference in the mean DD reading at one-month between the two groups ($P=0.032$). However, the mean DD readings were significantly lower in the n-HAP group at 3- and 6-months with high statistical significant level ($P<0.001$).

Conclusion: The nano-hydroxyapatite paste (nano-P) and the tri-calcium phosphate varnish (clinpro) were effective in WSLs' remineralization. Both treatment protocols could be advocated to promote remineralization of WSLs after orthodontic treatment. The nano-hydroxyapatite paste appears to demonstrate better stability than the tri-calcium phosphate varnish in its remineralization effect over the 6-month follow-up.

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INTRODUCTION

White spot lesions (WSLs) that appear as a sign of initial enamel demineralization is commonly seen nowadays especially after fixed orthodontic treatment. Enamel demineralization starts at atomic level on the surface of the hydroxyapatite crystals and precede to change enamel morphology developing an enamel lesion then dentinal lesion resulting in an obvious cavitated carious lesions if not treated (Goswami et al. 2012).

It is well established that dental caries in its early stage causes loss of the mineral components from enamel subsurface. Bacteria produce acids which in-turn lead to loss of a large amount of calcium and phosphate, resulting in an early subsurface enamel lesion. This process is reversible and can be completely halted if a proper remineralization protocol is applied, preventing further progression of the lesion and hence, improving strength, esthetics, and overall tooth performance. The remineralization process starts from saliva as it naturally supplies calcium and phosphate bioavailable ions to enamel. Saliva has a natural remineralization potential yet, still slow and rarely able to treat the lesion completely (Gugnani et al. 2012). External factors are therefore a prerequisite to supply calcium and phosphate ions to enhance ion deposition into voids of the demineralized enamel crystals resulting in mineral gain.

Fluoride is an essential adjunct in caries prevention. Fluoride is able to enhance remineralization in the presence of calcium and phosphate ions in saliva. Cochrane Oral Health Group stated that “a daily oral regimen incorporating at least 1,000 ppm fluoride likely provides the greatest anti-caries protection” (Walsh et al. 2009). High fluoride doses has been advocated for orthodontic patients in order to arrest decalcification (Trairatvorakul et al. 2008). However, one drawback is the rapid remineralization in the superficial layer of the WSLs which inhibit further penetration of calcium and phosphate ions to deeper enamel lesions (Jo et al. 2014). Likewise, several authors have raised concerns about using high fluoride concentration to assist in remineralization because of its toxicity potential and suggested that fluoride exposure should be limited when environmental sources of fluoride are present (Goswami et al. 2012; Huang et al. 2013). Therefore, additional highly efficacious technologies for enamel remineralization were envisioned along with their performance requirements.

In recent years, researchers have suggested a combination product supplying minimal concentration of fluoride with small amount of calcium and phosphate ions. Beta-tricalcium phosphate (β-TCP) was added to carboxylic acids and surfactants to obtain a “functionalized” tricalcium phosphate (f-TCP) (Karlinsey & Pfarrer, 2012; Rirattanapong et al. 2014). This f-TCP serves as a stable bioactive source of calcium and phosphate ions in a water-based fluoride-containing preparation that prevent the premature reaction between calcium and fluoride ions, thus permits a low dose delivery protocol that enhance fluoride-based nucleation activity. A previous clinical research showed that mouth-rinse containing F (225 ppm) and TCP produced a statistically significant higher remineralization potential compared to 225 ppm F alone or saliva only (Amaechi et al. 2010). This new formulation was licensed by (3M ESPE) for use in toothpaste and fluoride varnish based on a protective barrier formed around calcium during manufacturing process, once the varnish flows on tooth enamel and contact the saliva, the barrier is broken making fluoride, phosphate and calcium ions free to enhance the remineralization potential. It was suggested that minimal amount of functionalized TCP were needed to allow mineral nucleation without the negative interaction with fluoride ions (Rirattanapong et al. 2014).

Another emerging remineralizing agent is hydroxyapatite, bioactive material known of its biocompatibility. It is used in medicine as well as in dentistry (Tschoppe et al. 2011). In comparison to hydroxyapatite, nano-hydroxyapatite (n-HAP) showed strong affinity to the tooth, had a higher solubility, surface energy, bioactivity and its structure was found to be similar to dental apatite (Roveri et al. 2009; Haghgoo et al. 2014). Previous research reports indicated that n-HAP can be used as a reminaralizing agent due to the size of the nano-particles in relation to the enamel ultrastructure (Li et al.2008; Hannig et al. 2010; Tschoppe et al. 2011; Huang et al. 2011). There are numbers of dentifrices and mouth-rinse formulations containing n-HAP and were strongly recommended to patients with fluorosis, xerostomic patients and because of their potential to occlude dentinal tubules, may be useful in the treatment of dentin hypersensitivity. The preliminary evidence suggests that these new products may offer therapeutic options with increased quantity and quality of the remineralized apatite crystals. There are few in vitro studies investigated the remineralization potential of these new products. To date and up to our knowledge, there have been no reported clinical studies evaluated the effectiveness of nano-hydroxyapatite paste and tri-calcium phosphate in the treatment of WSLs. With the development of more refined devices of high sensitivity and ability to give accurate diagnostic information about early carious lesions, clinicians can now assess the level of demineralization of individual lesions and are able to use the remineralization technology and follow-up on the results in a predictable way. Therefore, the aim of this study was to evaluate and compare clinically the effect of a nano-hydroxyapatite paste (n-HAP) and a tri-calcium phosphate fluoride varnish (TCP-F) in remineralization of white spot lesions (WSLs) in young permanent teeth. The first null hypothesis to be investigated was that there was no difference in the WSLs status at baseline and after remineralization up to the 6-month period. The second null hypothesis was that there was no difference between n-HAP and TCP-F in the remineralization potential of WSLs over six-month follow-up period.

MATERIALS AND METHODS

Study design and participant eligibility

This randomized clinical trial was conducted at Beirut Arab University; Specialty Dental Clinics. The research protocol and study design were approved by the BAU-IRB (approval code 2017H-0051-D-R-0235). Sample size calculation was made using a free online calculator (www.sample-size.net) at a true level of significance of 5% with 95% level of confidence and based on a previous research (Brunton et al. 2013). Patients included in this study were between the ages of 12 and 16 years" years; had at least one pair opposing or contralateral teeth with obvious WSL on the facial surface (code 1 or 2 according to ICDAS), having normal salivary rate ($\geq$ 0.2 ml/min Unstimulated) and had initial DIAGNODENT PEN (DD) reading between 14-29 indicating enamel demineralization without dentin involvement. Patients with any abnormal oral, medical, or mental condition; taking medication that affect salivary flow, having any previous treatment for WSLs or any WSLs with obvious cavitation were excluded. Consent forms were signed by patients and/or their parents before participating in this clinical study. In accordance with the previously mentioned criteria, 20 eligible participants (12 males, 8 females) with a mean age of 14 were selected after patients screening. Accessible maxillary and/or mandibular teeth having WSL were selected provided that there were equal number of teeth with WSLs affecting enamel only in the opposing or contralateral side of the arch in the same subject. To that end, the total number of teeth included in this study was 80 teeth with WSLs in the facial or buccal surface of the teeth. For ethical reasons, excess number of WSLs present within the same subject received treatment but were not included in the statistical analysis.

MATERIALS

Materials and equipment used in the study are listed in table 1.

TABLE (1) Materials and equipment

Material/Equipment	*Abbreviation*	*Description*	*Manufacturer*
Desensibilize™ Nano P paste	n-HAP	Ca-nano-phosphate organized in crystalline HA K-nitrate, H_2O, surfactant, 9,000 ppm NF	FGM, Joinville, SC, Brazil
Clinpro™ White Varnish	TCP-F	5% NF, TCP	3M ESPE Dental Products-Ireland
Diagnodent pen	DD	laser induced fluorescence detection device	Kavo Dental – Germany

Intervention

Treatment was conducted by one operator at all time periods. Teeth were cleaned with a toothbrush and oral prophylaxis was performed using polishing paste and a brush prior to isolation with Optradam (Ivoclar), Liechtenstein). Excessive saliva was removed with cotton roll and teeth were dried with air syringe before DD assessment.

A baseline reading of demineralized WSL was recorded using a laser-induced fluorescence detection device (Diagnodent pen) prior to application of a n-HAP paste or TCP-F varnish. The device was calibrated as per manufacturer's instructions each time before use. Assessments were carried out by another operator at all follow-up visits, who was not informed about the treatment protocol of the teeth involved. For each tooth with WSL, the whole facial surface was assessed using the probe tip B of the DIAGNOdent pen in a pendulous slow motion to guarantee that it would pick up the reflected fluorescence from the WSL and its margin, where the demineralization often starts. For each tooth surface the highest reading was recorded from the displaying screen of the device. This process was repeated for three-times to guarantee consistency in readings.

This was a double-blinded study design in which patients were not aware which WSL was assigned to n-HAP or TCP-F reminaralization. For the n-HAP group, a micro-brush was used to apply the paste over the demineralized area. The material was rubbed on the tooth surface for 10 seconds using a felt disk. The product was left in contact with the tooth for 5 minutes then the excess was removed.

For TCP-F group, the varnish was carefully mixed in the container, painted with the varnish application brush evenly over the demineralized area then left in contact with the tooth. The subject was advised to refrain from food and drinks for 30 minutes after treatment.

The application procedures were repeated every week for four weeks then at one, three and six-month recall visits, DD readings were recorded at one-month, three and six-month follow-up visits. Subjects were provided with appropriate dentifrice and a toothbrush and were instructed to brush their teeth three-times daily for five minutes, in the morning, immediately after lunch, and the last thing before bed. Subjects were also requested not to use other oral hygiene products during the study to ensure consistency, and maintain their normal dietary habits.

Statistical analysis:

Statistical analyses were done using SPSS for Windows version 20.0 with an alpha error of set value of 0.05. Descriptive Data including mean and standard deviation of DD readings was presented. Kolmogorov-Smirnov and Shapiro-Wilk tests were used to assess if variables in different groups were normally distributed. Non-parametric tests were utilized for statistical comparisons since the variables were not normally distributed. Friedman and Wilcoxon tests were performed to compare the DD readings over time; at baseline, after one month, three months and six months for both groups.

RESULTS

Table (2) and figure (1) show the mean (±SD) of DD readings at baseline, after one, three and six-months follow-up visits for both groups.

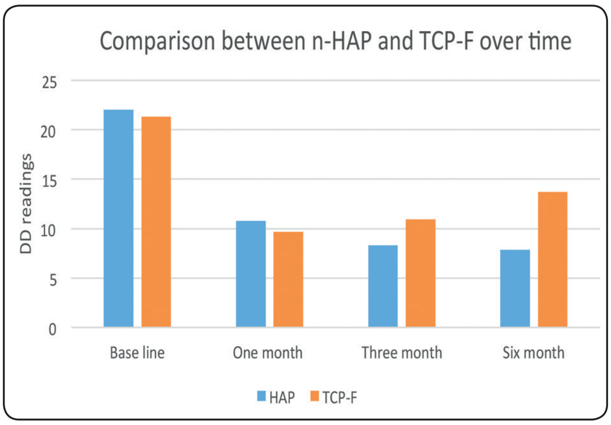

Fig. (1) Comparison between two groups at different time points

Comparison within the group:

The mean DD reading (±SD) of WSLs was 22.05 (±3.250) in n-HAP group and 21.33 (±3.467) in TCP-F group at baseline. After treatment, there was a significant decrease in the readings within each group at each time interval compared to baseline (P <0.001). This was decreased to 10.80 (±1.7423) and 9.675 (±2.693) at one-month, 8.30 (±1.880) and 10.95 (±2.726) at 3-month, 7.875 (± 1.555) and 13.725 (±2.745) at 6-month follow-visits in n-HAP group and TCP-F group respectively.

Comparison between three- versus six-months revealed no significant difference in the n-HAP group (P=0.549), while there was a significant increase in the DD readings in TCP-F group at six-month follow-up visit (P<0.001).

Comparison between the groups

In both n-HAP group, and TCP-F group, the WSLs showed similar mean DD reading at baseline (P>0.05). There was no statistically significant difference in the mean DD reading at one-month between the two groups (P=0.032). However, the mean DD readings were significantly lower in the n-HAP group at 3- and 6-months with a very high statistical significant level (P <0.001).

Figures (2-5) reveal photographs of a sample case at baseline, one, three and six-months after treatment. The demarcation of the WSLs was less obvious after treatment and the color was less intensified for both treatment protocols.

TABLE (2) Mean and standard deviation of DD readings for two groups over time

DD readings Groups	Baseline	1- month	3-months	6- months	p-value
n-HAP	22.05 (±3.250)	10.80 (±1.7423)	8.30 (±1.880)	7.875 (±1.555)	**<0.001; Friedman test**
TCP-F	21.33 (±3.467)	9.675 (±2.693)	10.95 (±2.726)	13.725 (±2.745)	**<0.001; Friedman test**
p-value	0.284; **(Wilcoxon test)**	0.032; **(Wilcoxon test)**	**<0.001; (Wilcoxon test)**	**<0.001; (Wilcoxon test)**	

Fig. (2) WSLs at baseline

Fig. (3) WSLs 1-month after treatment (the right side of the arch was treated by TCP-F and the left side of the arch was treated by n-HAP

Fig. (4) WSLs 3-months after treatment

Fig. (5) WSLs 6-months after treatment

DISCUSSION

Demineralization in enamel and development of WSLs is a well-known clinical problem that influence esthetics and patient satisfaction with their smile especially after fixed orthodontic appliances. According to the literature, its prevalence after orthodontic treatment is about 50% (Brown et al 2016). WSLs are very difficult to improve and if left untreated it can progress to develop carious lesions. Fluoride the traditional method, is known to be effective in reducing apatite dissolution by forming less soluble fluoroapatite. Products with Low fluoride concentrations have been suggested to enhance remineralization of decalcified enamel but are unable to reconstruct the lost mineral structure as in WSLs. Some researches were also against using high concentration fluoridated agents in treating obvious white lesions, as those products produce surface hypermineralization which inhibit further remineralization in the deep layers of the lesion (Huang et al. 2013; Lopatiene et al. 2016). Considering the importance of treating the demineralized enamel surface layer, this study was conducted to assess and compare the effectiveness of a non-fluoride-based (n-HAP) and a combined product (TCP-F) in reminaralization of WSLs in a randomized controlled clinical setting.

To achieve comparative results with high level of evidence, the study design was set so that each patient received the two treatment protocols and act as their own control. Thus standardization of oral environmental conditions and domestic oral hygiene measures were possible. It was found that enamel decalcification occurs more frequently in adolescence as they have limited power of self-governance and generally have poor oral hygiene. Therefore, this age group was selected.

In this study the tooth surface with WSLs was assessed by a DIAGNOdent pen. This device was proved to have sensitivity of 75% which is considered acceptable and specificity of 96% which is considered very high for smooth surface enamel caries analysis (Shi et al. 2001). The changes in WSLs was assessed through the reading values which is an estimate of the organic content and bacterial metabolites found in the lesion (Astvaldsdottir et al. 2010). DIAGNOdent pen emits infrared light that can be absorbed by organic and inorganic tooth material and the remitted fluorescence shows various scales on the digital screen between 0 and 99, where 0-13 indicates healthy tooth structure, 14-20 indicated superficial enamel lesion, 21-29 indicates deep enamel lesion and 30+ indicates dentin caries. Teeth included in the study fell in the range of 14-29 which eventually confirms no dentin involvement. The similar baseline DD readings made it possible to establish a comparison between the two groups after treatment.

The results of this study determined that both n-HAP paste and TCP-F varnish effectively enhanced remineralization of WSLs in enamel. The significant drop in the mean DD readings to less than (14) after treatment and at all-time points suggests a reversal of the early caries process to a healthy tooth structure. Both materials were able to effectively induce and maintain enamel remineralization, therefore the first null hypothesis was rejected. Based on the manufacturer information, each material has a different composition and hence, different mechanism for promoting remineralization in the damaged enamel lesion. With respect to n-HAP, the nanostructured hydroxyapatite exhibits high biomimetic characteristics because of their composition, structure, size, morphology, surface physical and chemical properties. N-HAP enhances remineralization of demineralized enamel as it delivers calcium, phosphate, and fluoride ions. These ions can be integrated in the enamel structure as hydroxyapatite crystals, fluorapatite, or calcium fluoride. Its remineralization potential depend on the nano-sized hydroxyapatite ability to penetrate deeply into the subsurface as a result of friction for 10-seconds forming a "reservoir-like" deposits of calcium and phosphate ions available for further remineralization for longer duration (Huang et al. 2011). The newly formed crystals therefore were probably able to fill and occlude the nano-spaces resulted from enamel decalcification and helped maintaining a state of super-saturation with respect to enamel minerals (Bevilacqua et al. 2016). This may provide a reasonable explanation regarding the stability of the DD reading over the six-months. In accordance to these findings, there are few research studies investigated formulation containing nano-sized apatite particles in toothpastes and mouthrinses and reported similar findings (Amaechi 2015). In accordance with our results, previous in vitro studies confirmed the protective effect of n-HAP paste and its ability to repair early enamel lesions (Najibfard et al. 2011; Medeiros et al. 2014; de Carvalho et al. 2014, Daas et al. 2018). Additionally, Swarup and co-authors concluded that n-HAP were able to reharden the softened enamel by gradual deposition of the mineral that precipitates and nucleates in the dark zone of demineralization thereby offering complete biomimetic regeneration of the lost enamel crystallites (Swarup et al. 2012). TCP-F varnish however, has a different mode of action. TCP-F varnish contains f-TCP combined with fluoride to promote fluoride-based nucleation activity, with further remineralization through calcium and phosphate ions present in both diet and saliva. TCP adheres to tooth surfaces due to its high affinity. Saliva activates the calcium compound carried by TCP, degrades the protective coating, releases calcium at the tooth surface, resulting in high fluoride and calcium bioavailability on the surface of the lesion followed by subsequent diffusion into the lesion, promoting remineralization. TCP-F creates relatively large, densely packed crystals. A previous in vitro study found that combining NaF and TCP in a simple aqueous solution generated a significantly greater surface and subsurface rehardening of WSL when compared to that attained when fluoride without TCP was used (Amaechi 2015).

The above mentioned evidence may explain why both materials were effective remineralizing agents in treating WSLs yet n-HAP was more effective in penetrating into the nano-spaces, building nano-crystals into the deepest demineralized areas, making it more stable and maintaining its remineralization over the six-month period. Therefore, the second null hypothesis was rejected.

Another interesting point was observed during the follow-up period was the esthetic improvement of WSLs. A possible explanation could be related to the difference in the refractive index between healthy and decalcified enamel. Enamel has a refractive index of 1.62, when enamel is decalcified the subsurface pores is filled with saliva or air with a refractive index of 1.33 and 1.0 respectively (Kidd and Fejerkaro, 2004). As the refractive index of the three medias differ, the light is scattered and the lesion appear opaque. When these pores are remineralized and filled with crystal deposition, the refractive index is further changed and the opacity of WSLs becomes less intensified; improving the esthetic outcome. This may explain the noticeable improvement in the degree of opacity of the WSLs after remineralization.

CONCLUSION

The nano-hydroxyapatite paste (nano-P) and the tri-calcium phosphate varnish (clinpro) were effective in remineralization of the WSLs. Both treatment protocols could be advocated to promote remineralization of WSLs after orthodontic treatment. The nano-hydroxyapatite paste appears to demonstrate better stability than the tri-calcium phosphate varnish in its remineralization effect over the 6-month follow-up.

RECOMMENDATION

Further studies with a longer observation period are recommended to verify whether this esthetic change in WSLs is sustained.

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