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COMPARISON BETWEEN NANO-HYDROXYAPATITE AND CPP-ACPF IN REMINERALIZING EARLY CARIOUS LESIONS (IN VITRO STUDY)

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White spot lesion, remineralization, mineral count, non-cavitated lesions, DIAGNOdent pen

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Abstract

Poor oral hygiene, bacteria and orthodontic appliances mainly lead to early demineralized lesions where early detection and diagnosis of the lesion is a must so that it can be assessed and cured with the least invasive treatment. The objective was to evaluate the effect of nano-hydroxyapatite (nano-HA) and casein phosphopeptide-amorphous calcium phosphate fluoride (CPP-ACPF) on early demineralized enamel using DIAGNOdent pen and energy dispersive X-ray analysis (EDX). **Materials and methods:** A total of 60 enamel specimens were divided into four groups (n=15), where Group A: experimental; treated with nano-HA tooth paste, Group B: self-control for group A, Group C: experimental; treated with CPP-ACPF paste, and Group D: self-control for Group C. Control specimens were treated with fluoride varnish. All specimens were exposed to artificial demineralization followed by application of the respective agent for each and stored in artificial saliva. For normally distributed data, comparison between more than two population were analyzed using F-test (ANOVA) and One way analysis of variance (ANOVA) was performed for comparison between more than two groups. The significance level was set at $p \leq 0.05$. **Results:** There was statistical significant difference regarding DIAGNOdent pen readings and mineral count of calcium and phosphate at different periods in all groups ($P < 0.05$). There was no statistical significant difference between groups A and B and between groups C and D regarding DIAGNOdent pen readings and mineral count of calcium and phosphate at all periods ($P > 0.05$). **Conclusion:** Nano-HA and CPP-ACPF had similar potential in remineralizing initial enamel lesion as fluoride varnish and can reverse a lesion into a sound tooth structure with higher net mineral gain. DIAGNOdent pen was found to be a non-highly specific and relevant tool in the assessment of enamel mineralization.

Keywords

White spot lesion, remineralization, mineral count, non-cavitated lesions, DIAGNOdent pen

1. INTRODUCTION

Minimally Invasive Dentistry (MID), known as minimal intervention dentistry and preservative dentistry, is an essential at the present time in the treatment of oral diseases. Mainly, it is involved in the treatment of carious lesions that are demineralized but not cavitated, which are now healed instead of removed. Moreover, to achieve MID in early lesions, some principles must be applied such as early detection and diagnosis of the lesion, then controlling the contributing factors followed by curing with the least invasive treatment. Most importantly, the treatment should be assessed and monitored to eliminate the risk of future demineralization and cavitation. However, if the lesion is already cavitated, then minimum intervention should be applied following the principle of repair rather than replacement of defective restoration and disease control (Yap, 2012).

Since teeth are in direct contact with saliva and oral cavity, they are affected by several factors including dental erosion, bacteria, carbohydrates and time. All these factors may lead to a complex phenomenon; non-cavitated lesions where a localized mineral loss from tooth surfaces occurs. This mineral loss leads to subsurface demineralization with an intact surface layer which eventually collapses into a full cavity if not controlled with time. However, this phenomenon could be arrested by the unique capacity of enamel, where the demineralized surface is flushed with minerals at neutral pH to restore a new surface on the existing crystal remnants in the subsurface lesion, leading to the natural repair of non-cavitated lesions (Grewal et al, 2017).

Since long time, several methods in tooth surface remineralization have been studied. These methods include various forms of remineralizing agents such as restorative materials, fissure sealants, chewing gums, mouth rinses and dentifrices. One of the most known and basically applied for caries prevention was fluoride (F). Nevertheless, some side effects had developed showing fluorosis due to the total fluoride intake. Therefore, fluoride alternatives have been proposed to avoid the previous concerns, including casein phosphopeptide stabilized amorphous calcium phosphate (CPP-ACP) and nano-hydroxyapatite (nano-HA) because of their anti-cariogenic characteristics (Ebadifar et al, 2017).

CPP-ACP is a milk derivative and considered to have anti-cariogenic properties due to the presence of casein, calcium (Ca) and phosphate (P). A newer concept was the incorporation of F into CPP-ACP to obtain casein phosphopeptide-amorphous calcium phosphate fluoride (CPP-ACPF). It has been shown that the content of this material is responsible for resistance to acid dissolution since casein phosphopeptide (CPP) of the CPP-ACPF complex can bind to enamel, biofilm and soft tissue, so that it delivers the Ca and P ions where they are needed to end by reforming the apatite crystals (Jayarajan et al, 2011).

The other alternative, hydroxyapatite (HA) had also received a great attention in medicine, dentistry and biology for the reason that it has similar characteristics to human hard tissue, biocompatibility and low solubility in humid environments. Basically, HA contains Ca and P crystals which are found in cementum, enamel and dentin; to be considered as one of the main components of tooth mineral content. One of the most important properties of HA is its antibacterial effect. Many studies have used HA for enamel lesion repair because of its chemical and structural similarity to tooth mineral content and evolved to the use of nanotechnology; nano-HA. It is believed that nano-HA have a higher efficacy to enhance remineralization of initial enamel and dental caries than HA due to its nano-size particles. Nano-HA has hydrophilic and wetting characteristics and is able to produce a thin but tightly bound layer on the tooth surface, resulting in a higher surface hardness and remineralization (Ebadifar et al, 2017).

In vitro technology has greatly evolved, for detection of the demineralized areas and caries on occlusal and proximal surfaces of the tooth; a more portable battery powered laser fluorescence device, DIAGNOdent Pen (KaVo) has been evolved. Laser fluorescence (LF) device is one of the most commonly used methodology in restorative dentistry, to be considered as a simple, quantitative and comparable method of evaluating the performance of the various techniques (Jayarajan et al, 2011, Uysal et al, 2009).

Another technology is the energy dispersive X-ray analysis (EDX) which enables a quantitative evaluation of the composition or the chemical elements on the tested surface (Roman et al, 2019).

It is hypothised that there is no difference in the surface topography and mineralization of early demineralized enamel that is treated with nano-HA versus CPP-ACPF.

2. MAIN TEXT

Material and Method

In the present study, 30 extracted sound premolar teeth with intact mesial and distal enamel surfaces were collected, sectioned bucco-lingual and coded 1-15 such that the mesial and the distal half of each tooth have the same number and different letter (1M and 1D). The obtained specimens (60) were divided randomly into four groups (n=15), with the following inclusion and exclusion criteria;

Inclusion-criteria

Sound premolar teeth with intact mesial and distal enamel surface were scheduled for extraction for orthodontic reasons in the age range of 16-30 years.

Exclusion-criteria

Teeth were examined by naked eye and under dental microscope (Leica microsystem), surfaces to be treated had to be free of:

- Carious lesion
- Cracks
- Wear
- Hypocalcification
- Restoration
- Fracture
- Fluorosis

Sample grouping:

- Group A (experimental):15 specimen of mesial halves were treated by Desensin repair toothpaste, (nano-HA).
- Group B (self-control of group A):15 specimen of distal halves of the same teeth of group A were treated by Clinpro White Varnish, (fTCP).
- Group C (experimental):15 specimen of mesial halves were treated by MI Paste Plus, (CPP-ACPF).
- Group D (self-control of group C):15 specimen of distal halves of the same teeth of group C were treated by Clinpro White Varnish, (fTCP).

Materials used in the study

Table 1: Materials used in the study

Material	Description	Manufacturer
Desensin repair toothpaste	Hydroxyapatite nano-particles 0.45%, Potassium nitrate 5.00%, Sodium mono-fluorophosphate 1.10%, Provitamin B5 1.00%, Allantoin 0.10%, Vitamin E 0.30%	DENTAID S.L. Spain www.dentaid.com
MI Paste Plus	Contains 0.2% (900ppm) Fluoride and Recaldent (CPP-ACP)	GC America, Inc www.gcamerica.com
Clinpro White Varnish	1 ml of Clinpro White varnish contains: 50 mg of sodium fluoride, corresponding to 22.6 mg of fluoride ions, in an alcohol based solution of modified resins.	3M ESPE www.3mespe.co.uk

Specimen preparation

The selected teeth were thoroughly cleaned of debris using fluoride-free, prophylactic pumice paste, rubber cups and a low-speed hand piece, and refrigerated in 0.1% thymol until being used (De Carvalho et al, 2014).

Mesial and distal surfaces of teeth were examined using DIAGNOdent pen. 60 specimens considered healthy were prepared from those surfaces which showed a reading less than 13, while surfaces showing reading more than 13 were excluded (Davari et al, 2019).

The selected teeth were sectioned bucco-lingually from the middle of cusp tip using a water-cooled diamond saw, and then mounted from the root portion in self-cure acrylic resin blocks in a rubber mould showing the crown only (Jayarajan et al, 2011).

The acrylic resin manufacturer instructions were followed in water-powder ratio, mixing time and setting time. After acrylic setting, teeth in acrylic resin blocks were removed from the rubber mould and polished. Each specimen was stored in labeled test tubes coded with a number (1-15) and letter (M or D), where M stands for mesial surface and D for distal surface (for example: 1M or 1D). After cutting the specimen, a polyvinyl stencil of 4x4 mm dimension was placed on the mesial and distal surfaces of the specimen. Then the specimen were coated with a transparent acid resistant nail varnish except for the 4x4 mm area of the polyvinyl stencil. After setting of the varnish, the stencil was removed leaving a window of 4× 4 mm on the specimen surface. This was the area of interest, as the only area which will be analyzed for the change in DIAGNOdent pen readings and EDX. Then the specimens were immersed individually in demineralizing solution for 96 hours at 37°C to produce artificial carious lesions without cavitation (Mehta et al, 2013).

Solutions Preparation

The demineralization solution was prepared as described by Watanabe et al. (2005), it consisted of:

2.2mM Calcium chloride ($CaCl_2$),

2.2mM Potassium phosphate (KH_2PO_4),

- 0.05M Acetic acid,
- 1M Potassium hydroxide (KOH) used to adjust pH to 4.4.

The remineralization solution was prepared as described by Rajan et al. (2015), it consisted of:

- 20mM Sodium carbonate (NaH_2CO_3),
- 3mM Sodium phosphate (NaH_2PO_4),
- 1mM Calcium chloride ($CaCl_2$),
- 0.15M Potassium chloride (KCl) used to adjust pH of 7.

Artificial Enamel Lesion Preparation

The specimens were immersed individually in 10ml of demineralizing solution for 96 hours at 37°C. Artificial lesions without cavitation were produced.

Remineralization Phase

Group A (Nano-hydroxyapatite group): specimens were brushed using a soft tooth brush with Desensin repair toothpaste for 30 cycles, 15 seconds each, twice daily. The brushing intervention lasted for 15 days, where the specimens were dried and stored in artificial saliva through the whole procedure (Ebadifar et al, 2017).

Group C (CPP-ACPF group): specimens were treated with MI Paste Plus in accordance to the manufacturer instructions using a micro-brush applicator for 3 minutes, twice daily for 15 days. Any remaining paste on the surface was left and the specimen were stored in artificial saliva.

Group B and Group D (fluoride varnish group, fTCP): specimens of these groups were used as positive self-control. Clinpro White Varnish was mixed according to the manufacturer instructions, where entire content of one unit-dose package was dispensed onto the shaded inner circle and mixed with brush to be applied to the tooth surface, specimens were then stored in artificial saliva, after which the varnish was carefully removed with a scalpel blade, taking care to avoid touching of the enamel surface.

The remineralizing solution (artificial saliva) was changed every 48 hours for all the groups to avoid depletion or saturation of the solution and accumulation of the enamel dissolution products (Rajan et al, 2015).

Evaluation Methods

- DIAGNOdent pen evaluation

The examination of the specimens was done using DIAGNOdent pen to quantify the white lesions objectively. As recommended by the manufacturer instructions, before every measurement session the instrument was calibrated against its own ceramic disk. The device provides a two types of probes; type A: used in pits and fissures and type B: used for smooth caries. During this study type B probe was used.

The specimens were placed horizontally with the lesion facing upward, which allowed the standardization of the measurement by holding the tip 90 degrees to the lesion surface.

Measurements were interpreted according to the manufacturer of the DIAGNOdent (Table 2). (Jayarajan et al., 2011). Measurements were done at 3 stages; baseline, after lesion formation and after the remineralization phase (post treatment). All specimen were dried before reading of DIAGNOdent in order not to cause any light reflection, where the peak score of the DIAGNOdent pen was recorded during surface assessment, and it was recalibrated every 10 records.

Table 2. DIAGNOdent values interpretation

0-13	Healthy Tooth Structure
14-20	Enamel Caries
21-29	Deep Enamel Caries
30+	Dentin Caries

- Energy dispersive X-ray analysis (EDX)

EDX was carried out to quantitatively evaluate the enamel mineral counts of the specimen including Ca and P. It was evaluated at three stages; at baseline, after demineralization and after remineralization.

Data were collected and statistically analyzed.

3. RESULTS

Result of DIAGNOdent pen readings:

DIAGNOdent pen readings in the four groups at different periods:

Mean, standard deviation and range in the readings of DAGNOdent pen for different remineralizing materials showed increase in mean values after demineralization, followed by decrease upon remineralization in all groups. There was statistical significant difference regarding DIAGNOdent pen readings at different periods in all groups ($P < 0.05$) (Table 3 and Fig. 1).

Table (3): DIAGNOdent pen readings in the four studied groups at different periods.

	Baseline	**Demineralization**	**Remineralization**
Group A: Experimental group; "Desensin repair" tooth paste			
Range Mean S.D.	4-13 6.4 2.1	8-30 13 5.4	3-10 6.33 2.2
ANOVA P	16.85 0.001*		
P1 P2 P3	0.0001* 0.466 N.S. 0.001*		
Group B: Positive control group; "Clinpro White Varnish"			
Range Mean S.D.	3.0-13.0 7.13 3.1	7.0-26.0 13.93 5.7	3.0-20.0 7.60 4.4
ANOVA P	19.25 0.001*		
P1 P2 P3	0.0001* 0.3691 N.S. 0.001*		
Group C: Experimental group; "MI Paste Plus"			
Range Mean S.D.	4.0-13.0 7.73 2.52	9.0-24.0 14.87 3.72	4.0-14.0 7.73 2.89
ANOVA P	20.95 0.0001*		

P1 P2 P3	0.0001* 0.50 N.S. 0.0001*		
Group D: Positive control group; "Clinpro White Varnish"			
Range Mean S.D.	4.0-10.0 6.33 1.42	5.0-22.0 13.61 3.42	5.0-12.0 7.01 2.90
ANOVA P	12.0 0.001*		
P1 P2 P3	0.003* 0.107 N.S. 0.001*		

P1 comparison between base line and demineralization
P2 comparison between baseline and remineralization
P3 comparison between demineralization and remineralization
P was significant if ≤ 0.05
* Significant difference
N.S. Not significant difference

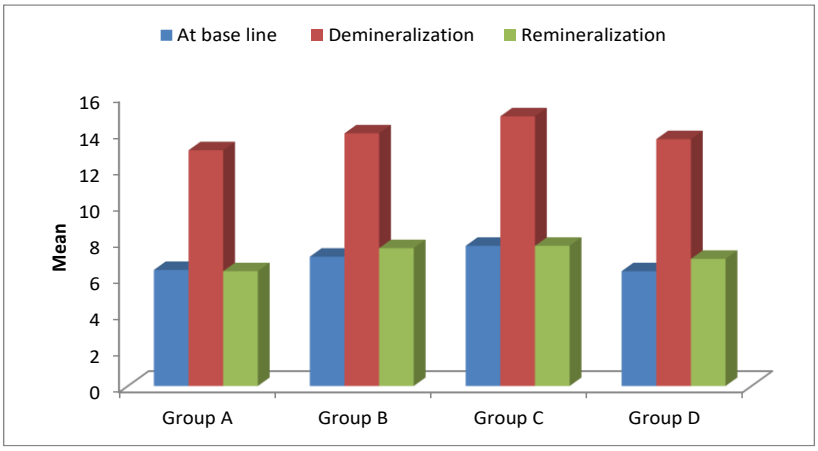

Fig.1: DIAGNOdent pen readings in the four studied groups at different periods

Results of EDX analysis, Ca and P mineral count:
Mineral content of calcium in the four groups at different periods:

In all groups (A, B, C and D), there was a statistical significant difference at all periods, showing decrease in calcium content after demineralization, and significant increase after remineralization ($P < 0.05$). There was a statistical significant difference between baseline and remineralization in all groups ($P<0.05$) (Table 4 and Fig. 2).

Table (4): Calcium in the four studied groups at different periods.

	Baseline	**Demineralization**	**Remineralization**
Group A: Experimental group; "Desensin repair" tooth paste			
Range Mean S.D.	18.33-35.2 26.12 7.01	16.38-31.0 20.11 6.02	19.66-37.33 29.11 7.25
ANOVA P	11.25 0.003*		
P1 P2 P3	0.025* 0.036* 0.001*		
Group B: Positive control group; "treated by Clinpro White Varnish"			
Range Mean S.D.	24.73-28.09 26.41 1.94	21.77-26.91 22.33 2.71	27.56-30.11 29.88 1.33
ANOVA P	7.11 0.003*		
P1 P2 P3	0.012* 0.006* 0.001*		
Group C: Experimental group; " MI Paste Plus"			

Range Mean S.D.	19.37-32.3 23.97 5.33	16.53-30.21 21.43 4.33	20.05-35.45 27.01 6.67
ANOVA P	8.71 0.0207*.		
P1 P2 P3	0.037* 0.021* 0.0061*		
Group D: Positive control group; "Clinpro White Varnish"			
Range Mean S.D.	22.3-26.18 24.24 2.24	20.11-25.84 22.01 3.05	24.67-29.88 26.52 4.01
ANOVA P	6.78 0.022*		
P1 P2 P3	0.0137* 0.025* 0.0061*		

P1 comparison between base line and demineralization
P2 comparison between baseline and remineralization
P3 comparison between demineralization and remineralization
P was significant if $\leq$ 0.05
* Significant difference
N.S. Not significant difference

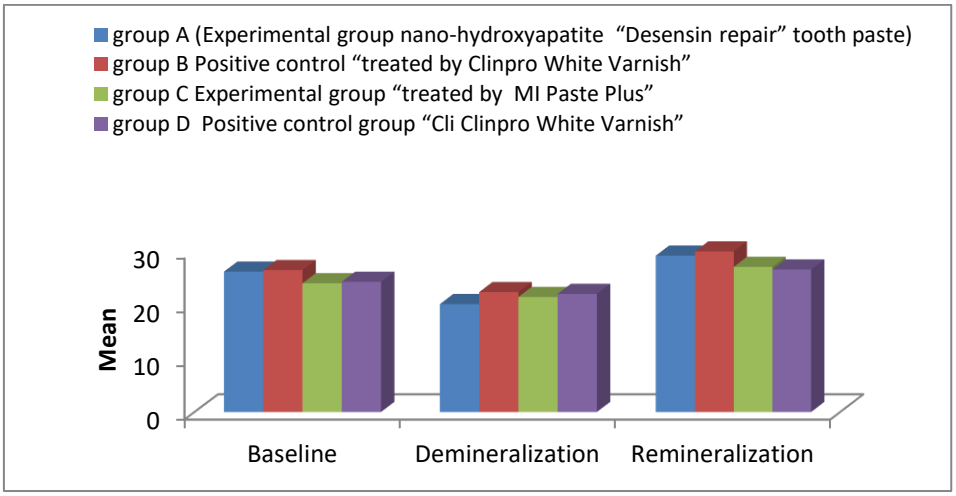

Fig.2: Calcium in the four studied groups at different periods.

Mineral content of phosphate in the four groups at different periods:

In all groups (A, B, C and D), there was a statistical significant difference at all periods, showing decrease in calcium content after demineralization, and significant increase after remineralization ($P < 0.05$). There was a statistical significant difference between baseline and remineralization in all groups ($P<0.05$) (Table 5 and Fig. 3).

Table (5): Phosphorus in the four studied groups at different periods.

	Baseline	**Demineralization**	**Remineralization**
Group A: Experimental group; "Desensin repair" tooth paste			
Range Mean S.D.	10.8-16.82 14.08 2.86	8.84-15.74 12.11 2.21	11.65-18.01 16.31 2.14
ANOVA P	6.55 0.0223*		
P1 P2 P3	0.026* 0.0160* 0.0105*		
Group B: Positive control group; "Clinpro White Varnish"			
Range Mean S.D.	13.27-15.82 14.55 1.47	11.49-13.82 12.06 1.09	14.92-17.67 15.95 0.72

ANOVA P	5.95 0.0371*		
P1 P2 P3	0.0442* 0.021* 0.010*		
Group C: Experimental group; " MI Paste Plus"			
Range Mean S.D.	10.96-16.1 12.55 2.39	9.36-15.22 11.37 2.11	11.1-17.81 14.11 2.67
ANOVA P	10.36 0.0205*		
P1 P2 P3	0.0368* 0.0212* 0.007*		
Group D: Positive control group; "Clinpro White Varnish"			
Range Mean S.D.	11.89-14.1 13.00 1.28	10.1-13.93 12.17 2.11	12.93-15.89 14.72 1.65
ANOVA P	6.151 0.036*		
P1 P2 P3	0.029* 0.041* 0.019*		

P1 comparison between base line and demineralization
P2 comparison between baseline and remineralization
P3 comparison between demineralization and remineralization
P was significant if ≤ 0.05
N.S. Not significant difference

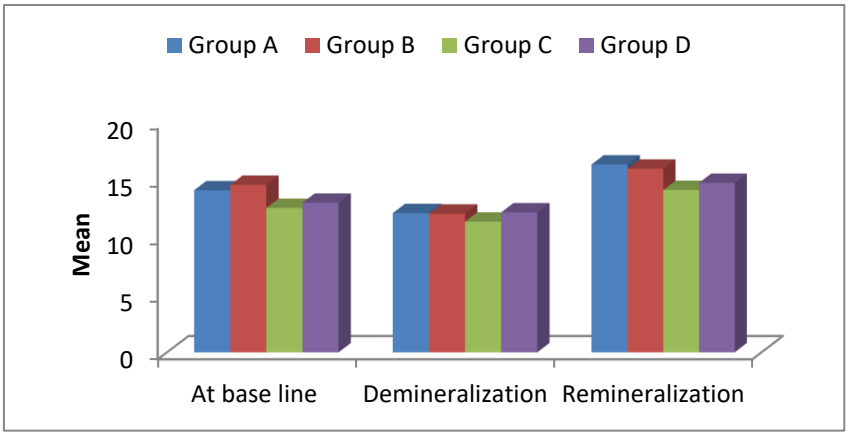

Fig.3: Phosphorus in the four studied groups at different periods.

4. DISCUSSION

A greater understanding of early carious lesions and its predisposing factors such as saliva, poor oral hygiene from orthodontic treatment, carbohydrates, bacteria, dental erosion and time; provoking factors in tooth mineral loss and subsurface demineralization with an intact surface layer, had challenged the practicing dentists to increase the importance of preventive and minimal intervention dentistry coupled with the established higher prevalence of non-cavitated caries. Despite the natural ability of saliva to remineralize enamel by supplying calcium and phosphate ions to the tooth at physiological pH, researchers have found that the net mineralization by saliva is small and appeared to be limited mainly to the surface layer of the lesion. Therefore, researches stated that new remineralization systems would be needed to mediate and control the remineralization process (Jefferies SR, 2014).

Recent investigations and new products focused on effect of calcium phosphate-based compostions, and others of nano-HA particles on initial enamel lesions. The aim of the current study was to compare between the remineralizing potential of nano-HA and CPP-ACPF on early carious lesions by considering fluoride containing varnish as a positive self –control.

The evaluation methods used in this study were DIAGNOdent pen and EDX. The DIAGNOdent pen was used to aid in the selection of caries-free, sound enamel surfaces, to ensure demineralization occurred before subjecting samples to EDX, and to detect and compare the changes before and after the application of the remineralizing agents. The other evaluation method used in this study was EDX analysis, it's purpose was to assess the mineral count of tooth structure, mainly Ca and P at three intervals; baseline, after demineralization and after remineralization. The aim of baseline measurements was to indicate normal mineral count of sound tooth structure and to aid in the evaluation of remineralizing potential of the remineralizing agents if they could return same mineral count of sound enamel before being exposed to demineralization.

Results of DIAGNOdent pen readings comparing groups at different periods revealed a significant increase in the mean values of DIAGNOdent scores after demineralization, followed by a significant decrease in mean values of DIAGNOdent scores after remineralization in all groups. These results affirmed that a difference in the fluorescence value had occurred distinguishing between healthy and demineralized dental tissue. However, there was no significant change in mean values of DIAGNOdent scores after remineralization compared to baseline. Thus, the application of either products is associated with significant enamel remineralization similar to the dental tissue before being exposed to demineralization. Results of comparison between nano-HA group and fTCP group, and between CPP-ACPF group and fTCP group regarding DIAGNOdent pen readings at same time revealed that there was no significant difference at all periods ($P > 0.05$). This indicates that all samples were selected with similar DIAGNOdent pen reading value at baseline. Furthermore, the demineralizing solution used in this study produced a standardized uniform artificial enamel lesion and made it possible to establish objective comparison among the groups.

After remineralization, the non-significant difference between the groups affirms that nano-HA and CPP-ACPF had similar effect to fTCP in remineralizing initial enamel lesion. So, as a result both agents have similar effectiveness in remineralization.This was in accordance with Patil et al. (2013) where they evaluated the remineralizing potential of CPP-ACP, CPP-ACPF and fTCP on artificially demineralized human enamel by using DIAGNOdent and E-SEM as evaluation methods. Their results showed a significant increase in the mean values of DIAGNOdent scores after demineralization, followed by a significant decrease in mean values of DIAGNOdent scores after 7 days of remineralization in all three remineralizing agents. This proves that CPP-ACP, CPP-ACPF and fTCP were all effective in remineralizing artificial enamel caries, which was similar to the results found in this study. In addition to this, Kamath et al. (2017) compared the remineralizing potential of fTCP, fluoridated dentifrice, CPP-ACPF and nano-HA using DIAGNOdent, SEM and EDX as evaluation methods. There was a statistical significant increase in the readings of DIAGNOdent post demineralization and a significant decrease post remineralization approaching baseline values for each of the experimental groups. Results revealed a non-significant difference among the remineralizing agents at different experimental stages. This affirms that all tests agents were comparable in remineralizing potential and that DIAGNOdent has the potential to be used as a non - invasive diagnostic and monitoring tool during remineralization therapies. Shen et al, (2015) revealed that ACP containing varnishes had significantly higher 24 hours release of Ca, inorganic P and F ions than fTCP varnishes, however it was unknown if the deposited fluoride was precipitated or incorporated into enamel crystal structure. On the other hand, Bandekar et al, (2019) reported that fTCP provides a Ca and P release system obtained by milling beta-calcium phosphate with sodium lauryl sulfate. This ensures that the calcium oxides are protected from unwanted interactions with fluoride, which could render both Ca and F inactive preventing Ca-P reaction with F and formation of calcium fluoride. Hence, Ca and P are available in an aqueous form for the remineralization process. Furthermore, the use of low F concentration as found in CPP-ACPF such as Clinpro tooth cream product helps to maintain a state of supersaturation by suppressing the demineralization, indicating the long term use of this product could remineralize enamel surface specifically in treatment of white spot lesions. This F incorporation into CPP-ACP leads to a synergistic anticariogenic effect resulting in the adsorption of F ions onto the surface of enamel crystals, preventing dissolution and increasing remineralization (Brar et al, 2017). Rodrigues et al. (2017) evaluated the performance of laser fluorescence pen (DIAGNOdent pen) in the detection of caries-like lesions' progression, and found that it was significantly effective in quantifying the initial enamel demineralization after the first cycle only; where there was a significant increase in the fluorescence values similar to this study.

They found that laser fluorescence pen (DIAGNOdent pen) didn't accurately measure small changes in the mineral content, and it was not effective in monitoring enamel lesion progression. Furthermore, Kavvadia et al. (2018) found that fluorescence devices over estimated mild and extended white spot lesion as compared with reference method (visual diagnostic methods). Based on this, it was stated that fluorescence devices had low sensitivity but good specificity and accuracy for mild lesions, but had higher sensitivity, accuracy and similar performance as direct visual methods in more extended lesions. They revealed that fluorescence device are not better than visual diagnostic methods in diagnosis and quantification of non-cavitated white spot lesion.

Results comparing the mineral content of Ca and P in the enamel specimens using EDX at different periods revealed a significant decrease in the mineral content after demineralization followed by a significant increase after application of the remineralizing agent in all groups. Moreover, after application of the remineralizing agent, all groups had a significant higher mineral content of Ca and P than baseline. This affirms that all agents could remineralize initial enamel lesion and enhance its mineral content leading to a harder dental tissue. Since the results of EDX revealed a significant higher value of mineral content after remineralization compared to baseline, while the results of DIAGNOdent pen reading showed non-significant difference, this proves that DIAGNOdent pen is a non-relevant and non-reliable tool. As mentioned before, the DIAGNOdent is a device that works by capturing fluorescence emitted by the oral bacteria prophyrins and other chromophores present on the demineralized dental tissue but does not indicate amount of demineralization. This coincides with Diniz et al. (2015) who evaluated the effectiveness of fluorescence based- methods and concluded that fluorescence devices could help in distinguishing between sound and demineralized enamel, where DIAGNOdent pen device had a better performance in indicating deep non-cavitated carious lesions.

This result could be justified by the presence of bacterial model for caries generation in their study, where it is considered to be more realistic than chemical systems as the artificial demineralizing solution used in the recent study. They stated that the device have the ability to identify bacterial metabolites such as prophyrins produced by cariogenic bacteria, which was proved by lower values for sensitivity and specificity of fluorescence devices after first cariogenic challenge, whereas after the second challenge the values were greater. Moreover, results of EDX comparing between nano-HA group and fTCP group and between CPP-ACPF group and fTCP group regarding Ca and P content revealed a non-significant difference at all periods ($P > 0.05$). This indicates that all samples were selected with similar mineral content at baseline. Furthermore, the demineralizing solution used in this study produced a standardized uniform artificial enamel lesion and made it possible to establish objective comparison among the groups. After remineralization, the non-significant difference between the groups affirms that nano-HA and CPP-ACPF had similar effect to fTCP in remineralizing initial enamel lesion. So, as a result both agents have similar effectiveness in remineralization. Similar results were found by Vijayasankari et al. (2019) who analyzed the remineralizing potential of CPP-ACP paste and nano-HA pastes of different concentrations by using SEM and EDX as evaluation methods. The similarity was in the significant reduction in Ca and P after demineralization and significant increase in Ca and P after remineralization in all groups except the control. This indicates that these materials had a positive effect on remineralization of early enamel caries. However, the mean Ca and P ratios post remineralization where significant in group treated with 10% nano-HA. These findings were in contrast with results of present study, where there was no significant difference between the groups, which might be due to the different concentration of the nano-HA used in this study. In the present study, all groups had a significant higher values of Ca and P than baseline which was in accordance with Davari et al. (2019) who found that 10% nano-HA helps in protection from lesion formation and aids in strengthening of the tooth structure, except that the other tested groups; (nano-HA group with less concentration, CPP-ACP group, white spot lesion group and healthy group) revealed less amount of Ca and P in contrast to the current study due to different concentration of nano-HA as mentioned before, in addition to the pH cycling procedure which was not applied in the current study. Zenouz et al. (2015) evaluated the effect of NaF gel, CPP-ACP and CPP-ACPF on enamel surface microhardness after microabrasion and found that the incorporation of F in CPP-ACPF does not provide any additional remineralizing potential when compared to CPP-ACP paste. This was in accordance with the present study where nano-HA and CPP-ACPF had similar effect to fTCP in remineralizing initial enamel lesion.

In the end, even though the in vitro model design of the lesion formation in this study mimics the mineral loss and gain involved in early carious lesion, a possible limitation of this study might be the absence of a bacterial model which simulates the biological oral conditions, and the 15 days of remineralizing agent application might not be enough in comparison of remineralizing efficacy.

Thus, the null hypothesis of this study was accepted were it was proved that there is no difference in the remineralizing efficacy of early demineralized enamel that is treated with nano-HA versus CPP-ACPF.

5. CONCLUSION

A. Nano-HA and CPP-ACPF had similar potential in remineralizing initial enamel lesion as fluoride varnish
B. Nano-HA and CPP-ACPF can reverse a demineralized lesion into a sound tooth structure with higher net mineral gain in Ca and P.
C. DIAGNOdent pen is not a highly specific and relevant tool in the quantitative assessment of enamel mineralization, it is a bacterial-mode dependant tool.

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