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ORIGINAL ARTICLE

Effect of different remineralizing agents on the initial carious lesions – A comparative study

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KEYWORDS

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Tri-calcium phosphate

Abstract *Objectives:* This study aimed to assess and compare the effectiveness of three different remineralizing agents (Tricalcium phosphate paste, Fluoride varnish, and Nano-hydroxyapatite gel) using the DIAGNOdent device.

Material and Methods: The present clinical study was carried out on 90 initial carious lesions detected by ICDAS caries diagnostic criteria and then take the baseline record by DIAGNOdent device. The selected initial carious lesions were randomly classified into three groups according to treatment modalities (30 lesions in each group) according to remineralizing agents: group A (TCP), group B (fluoride varnish) and group C (nano-hydroxyapatite gel). The remineralizing agents were applied for four minutes once weekly for four weeks. At the fifth week, the DIAGNOdent scores of initial carious lesions were recorded to evaluate the effect of remineralizing agents. A paired t-test was used to compare between baseline date and follow up of DIAGNOdent scores. A one-way ANOVA test was used to compare DIAGNOdent scores among the three groups. Post-Hoc Tukey test was used to determine the significant difference between every two groups.

Results: There were statistically significant differences among the three groups at follow up ($p = 0.001$). Within each group, there was a significant difference between baseline and follow up scores ($p = 0.000$ for the three groups). Multiple comparisons between every two groups showed a highly statistically significant difference at follow up records between nano-hydroxyapatite versus TCP and fluoride varnish on pit and fissure caries ($p = 0.039$ and $p = 0.007$ respectively) and the nano-hydroxyapatite was the best of them.

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Conclusion: The present study concluded that the three remineralizing agents were effective in the treatment of initial carious lesion and the most effective remineralizing agent was nano-hydroxyapatite.

1. Introduction

Dental caries is one of the most prevalent diseases worldwide. Due to their dynamic nature, the timing is very specific, and controlling caries is contingent on the remineralization of initial carious lesions (Rao and Malhotra, 2011; Somasundaram et al., 2013).

Remineralization refers to the treatment for an active initial carious lesion, by stopping the progression of the lesion to cavitated stages (Ismail et al., 1992). Researchers are seeking many pathways to minimize dental caries by trying to use a number of remineralization agents (Jayarajan et al., 2011).

Fluoride is the most common remineralization agent. More specifically, (gels, varnishes, and other fluoride-releasing materials) are highly effective at not only decreasing demineralization, but also increasing the remineralization of tooth structure when maintaining oral hygiene instructions (Mitchell, 1992; Derks et al., 2004).

Numerous studies have examined the differences between various remineralizing agents or made attempts to determine the best option. (Liena et al., 2015) carried out a prospective study to evaluate the effects of casein phospho-peptide - amorphous calcium phosphate (CPP-ACP) and CPP-amorphous calcium fluoride phosphate (CPP-ACFP) versus fluoride varnish on the remineralization of enamel white spot lesions (WSLs). According to the findings, the CPP-ACFP performs rapid remineralization as compared to other groups but, although there is no significant difference among them after three months.

Molaasadolah et al. (2017) conducted a study to evaluate enamel microhardness following the application of two types of fluoride varnish. Their results indicated that the use of fluoride significantly increased the enamel hardness.

Huang et al. (2009) studied the use of nano-hydroxyapatite-containing toothpaste and oral rinses tailored to be remineralizing agents as well as for preventing caries. As per the findings, the nano-hydroxyapatite had a remineralizing effect on initial enamel carious lesions.

Patil et al. (2013) performed an in vitro study to compare CPP-ACP, CPP-ACFP, tricalcium phosphate fluoride (TCP-F), and artificial saliva using DIAGNOdent and an electron microscope. All agents were found to have a significant remineralizing effect with the exception of artificial saliva compared with baseline scores.

In an in vitro study conducted to evaluate the effectiveness of tricalcium phosphate (TCP), fluoridated dentifrice, CPP-ACFP, and nano-hydroxyapatite in treatment of initial carious lesions. (Kamath et al., 2017) propounded that all agents showed remineralization effects and that there were no significant differences between them.

DIAGNOdent is a laser device that measures fluorescence and improves the diagnostic accuracy of detection of non-cavitated carious lesions (Braun et al., 2000). Thus, when used in the early stages, it can facilitate the application of preventive measures sufficiently early (Lussi et al., 2004).

From the previous studies, it can be inferred that the effectiveness of different remineralizing agents is somewhat controversial, and that most of the previous studies evaluated the effectiveness of remineralizing agents in vitro. As such, due to the continuous development of new remineralizing agents and scarcity of in vivo studies, research is needed to investigate the newer remineralizing agents under different circumstances. The present study was carried out to assess and compare the effectiveness of three different commercially available remineralizing agents (TCP, Fluoride varnish, and Nano-hydroxyapatite gel) in vivo.

2. Material and methods

2.1. Remineralizing agents used in this study

Three commercially available remineralizing agents were used for this study:

- Fluoride varnish 25 ml (5% NaF) – BELAK-F (VladMiVa, Russia).
- TCP paste –Clinpro™ (3 M ESPE, USA).
- Nano-Hydroxyapatite gel – CTx4 Gel 1100 (CariFree, Canada).

2.2. Ethical approval and trial design

The present study was an in vivo clinical trial, conducted at the dental clinic of the Dental Teaching Hospital in Umm Al-Qura University, Saudi Arabia after securing the ethical approval (IRB number 72-17) from institutional ethical committee. The trial consisted of three groups of patients with initial carious lesions, and were named according to the remineralizing agent used.

2.3. Sample size

The initial carious lesions were the specific target of this study. The sample size was calculated at confidence level 95%, confidence interval of 5% and expected percentages of caries prevention of 70%. The estimated sample was 84 which subsequently increased to 90 lesions.

2.4. Participant inclusion criteria

– Adult patients aged 20–40 years, free from the systemic diseases of Sjogren's syndrome, mumps, diabetes and –
hyperacidity.

– Patients with fair oral hygiene (mild to moderate plaque accumulation).
– Patients with non-cavitated initial carious lesions grade 1 or 2 caries in accordance with The International Caries Detection and Assessment System (ICDAS) caries diagnostic criteria (Sebastian and Johnson, 2015).
– Patient did not receive any remineralizing agent other than the regular toothpaste during the past three months.

2.5. Participant exclusion criteria

– Patients with glass ionomer restoration because the glass ionomer restoration release fluoride in the oral cavity, potentially impacting the results.

2.6. Randomization

The selected initial carious lesions were randomly classified into three groups according to treatment modalities as follows:

– Group A (TCP group).
– Group B (Fluoride varnish group).
– Group C (Nano Hydroxyapatite group).

Every participant received only one treatment modality. Each material was used to treat 30 initial carious lesions. The three treatment modalities were written on 90 cards (30 for each material), after which the patient was asked to pull a card randomly from a basket under the supervision of the researcher.

2.7. Blinding

Well-trained and calibrated examiner who was blind to the study design and materials performed DIAGNOdent scoring at baseline and at different follow-up. Additionally, the collected data was analyzed by an external statistician.

2.8. Data validity

The stability of measurements was confirmed at the baseline and during different follow-up. The intra-examiner consistency of measurements was confirmed before and during the study through training and calibration of the same evaluator. This calibration was tested by the Kappa test, following which the intra-examiner consistency was found to be 88%.

The calibration of DIAGNOdent in accordance with manufacture information:

- "Press the menu key (The calibration symbol appears).
- Press the save key (Calibration is started).
- Once you hear the signal, place the probe vertically on the reference (calibration disk).
- The signal tone stops, which means that the calibration is over.
- Calibration is successful when the value displayed agrees with the reference value 89 (±3)"89, the calibration number of DIAGNOdent device". Table 1 below depicts the DIAGNOdent measurements.

Table 1 DIAGNOdent measurements according to manufacture information.

Caries site	Healthy tooth substance score (1)	Beginning demineralization score (2)	Strong demineralization score (3)
Pits and fissure caries	0–12	13–24	>25
Smooth surface caries	0–7	8–15	>16

2.9. Clinical interventions

The selection of initial carious lesions was premised on the ICDAS caries diagnostic criteria. The DIAGNOdent baseline scores were recorded and only carious lesions with a score of 2 were included in the present study. The different remineralizing agents were applied to treat initial carious lesions according to the manufacturer's instructions for each material. Low-speed handpiece was used to clean the surface with fluoride-free pumice and cotton roll isolation.

2.9.1. Fluoride varnish application

A thin layer of the varnish was applied to the initial carious lesion for a minute until set. The patient was instructed to avoid abrasive diet for the rest of the day and not brush or floss until the next morning.

2.9.2. TCP application

The entire contents of the individual container were applied to the dark inner circle on the foil wrap of the dosing guide, the material was keeping inside the inner circle. The varnish was applied evenly to the areas to be treated and avoid moving the applicator brush through accumulated saliva to prevent premature setting on the bristles. The patient can eat and drink following the application.

2.9.3. Gel 1100 application

A thin layer of the gel was applied to the initial carious lesion for 4 min then wash it. The patient was instructed to eat and drink after 30 min.

Each remineralizing agent was applied once every week for a period of four weeks. Every patient in all three groups was educated and motivated about the importance of oral hygiene and tooth brushing with regular toothpaste contain NaF (1400 PPM). The DIAGNOdent was used to evaluate the remineralizing effect of different remineralizing agents at the end of the fifth week.

2.10. Statistical analysis

The collected data were analyzed using a Statistical Package for the Social Sciences software program (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp). For each group, the descriptive data analysis included the mean and standard deviation for baseline and follow-up data of the DIAGNOdent scores. The frequency distribution of pit and fissure caries and smooth surface among the three groups

was tested with Chi square test prior to intervention in order to ensure that no significant difference is found among the three groups. Paired *t* test was used to compare the baseline and follow-up scores for each group. In addition, one-way ANOVA test was used to make a comparison among all three groups and to determine whether there were any significant differences regarding baseline, and follow-up of the DIAGNOdent scores. Post- Hoc Tukey test was used to compare the two remineralizing agents for the baseline and follow-up records in order to determine the most effective agent. The level of significance was considered statistically significant at $p \leq 0.05$.

Table 2 The distribution of carious lesions among the three groups.

Groups	Pits and fissure initial caries no (%)	Smooth surface initial caries no (%)	Total
Group A	20 (67%)	10 (33%)	30 (100%)
Group B	19 (63%)	11 (37%)	30 (100%)
Group C	21 (70%)	9 (30%)	30 (100%)
Total	60 (67%)	30 (33%)	90 (100%)
P	0.837		

No = Number, group A = TCP, group B = Fluoride varnish, group C = Nano-hydroxyapatite, p = p value which calculated by Chi square test.

Table 3 The effect of different remineralizing agents on initial carious lesions.

Groups	Baseline Mean ± SD	Follow up Mean ± SD	P
Group A	16.00 ± 3.714	10.37 ± 3.37	0.000*
Group B	16.90 ± 3.633	10.60 ± 2.74	0.000*
Group C	15.50 ± 3.58	7.80 ± 3.13	0.000*
One-way ANOVA	0.311	0.001*	

Group A = TCP, group B = Fluoride varnish, group C = Nano-hydroxyapatite, SD = Standard Deviation, p = p value calculated by paired *t* test, * = statistically significant values, the numbers of Mean ± SD represent DIAGNOdent scores.

3. Results

No statistically significant difference was found among the three groups regarding the numbers of pits and fissures and smooth surface initial carious lesions (p = 0.837) (please refer to Table 2).

A comparison of the effect of different remineralizing agents revealed that there were no significant differences among the three groups as regards the DIAGNOdent baseline scores (p = 0.311). In contrast, statistically significant differences were found between them at follow-up (p = 0.001) [One-way ANOVA test]. Within each group, statistically significant differences were found between baseline and follow-up scores (p = 0.000 for the three groups) [paired *t* test] Table 3 illustrates depict these differences.

A comparison of the effect of remineralizing agents on different tooth surfaces indicated that there were no significant differences among the three groups with regard to the DIAGNOdent baseline scores of pits and fissures (p = 0.053) as well as smooth surfaces (p = 0.602). On the other hand, statistically significant differences were found among them for pits and fissure scores (p = 0.006) at follow-up, but no significant differences were observed for smooth surfaces (p = 0.058). Within each group, statistically significant difference was found between baseline and follow-up scores (p = 0.000 for all comparisons) [paired *t* test], as shown in Table 4.

Multiple comparisons between every two groups showed no significant differences at the baseline. At follow-up, a highly statistically significant difference was found between nano-hydroxyapatite versus TCP and fluoride varnish on pit and

Table 4 The effect of different remineralizing agents on initial carious lesions of different surfaces.

Groups	Pits and fissure initial caries			Smooth surface initial caries		
	Baseline Mean ± SD	Follow up Mean ± SD	P	Baseline Mean ± SD	Follow up Mean ± SD	P
Group A	17.35 ± 3.66	10.85 ± 3.50	0.000*	13.30 ± 2.00	9.40 ± 3.03	0.000*
Group B	18.89 ± 2.83	11.53 ± 2.67	0.000*	13.45 ± 1.81	9.00 ± 2.09	0.000*
Group C	16.24 ± 3.55	8.33 ± 3.35	0.000*	14.44 ± 3.88	6.67 ± 2.45	0.000*
One-way ANOVA	0.053	0.006*		0.602	0.058	

Group A = TCP, group B = Fluoride varnish, group C = Nano-hydroxyapatite, SD = Standard Deviation, p = p value calculated by paired *t* test, * = statistically significant values, the numbers of Mean ± SD represent DIAGNOdent scores.

Table 5 Multiple comparisons between every two groups.

Multiple comparisons	Pits and fissure initial caries		Smooth surface initial caries		Total effect	
	Baseline P	Follow up P	Baseline P	Follow up P	Baseline P	Follow up P
A VS B	0.334	0.788	0.990	0.931	0.594	0.954
A VS C	0.546	0.039*	0.619	0.067	0.851	0.005*
B VS C	0.420	0.007*	0.687	0.121	0.288	0.002*

A = TCP group, B = Fluoride varnish group, C = Nano-hydroxyapatite group, P = P value calculated by Tukey test, *= statistically significant values.

fissure caries ($p = 0.039$ and $p = 0.007$, respectively) as well as a total effect ($p = 0.005$ and $p = 0.002$ respectively). Meanwhile no significant difference was found between TCP and fluoride varnish on fissure caries. In addition, no significant differences were found for the smooth surface follow-up, as displayed in Table 5.

4. Discussion

The continuous improvement and innovation of remineralizing agents, coupled with their increased availability in the market have resulted in several in vivo clinical trials to select the most effective one for the treatment of initial carious lesions. Against this backdrop, the present study was carried out to evaluate and compare the remineralization effectiveness of three different commercially available remineralizing agents for the treatment of initial carious lesions.

In the present study, the DIAGNOdent device was used to evaluate the remineralizing effect because it is an objective accurate method of detecting initial carious lesions as compared to the visual examination (Lussi et al., 2004; Moriyama et al., 2014). The finding of the current study indicated that the three remineralizing agents have a significant remineralizing effect on initial caries on both occlusal and smooth surfaces. It was found that the fluoride varnish and TCP have similar remineralizing effects in treating initial caries. These results are in consonance with the findings of (Patil et al., 2013).

The remineralizing effect of fluoride varnish in the present study was found to be highly significant in the treatment of initial caries, which is in line with the results obtained by (Singh et al., 2016) and (Molaasadolah et al., 2017). Meanwhile fluoride varnish acts as a reservoir of slowly releasing fluoride ions. In other words, fluoride inhibits enamel demineralization and promulgates remineralization. Whether or not fluoride remains on the enamel and subsurface lesion is primarily contingent on the presence of calcium and phosphate ions (Babu et al., 2018).

The significant remineralizing effect of TCP can be explained by increased calcium levels in plaque and saliva after its application, the presence of free calcium and phosphorus ions, as well as the presence of fluoride ions in saliva, which provided a suitable remineralizing solution to facilitate the remineralization of initial carious lesions. These results are in line with the findings of (Bajaj et al., 2016), who use functional TCP milled with sodium lauryl sulfate in order to treat initial carious lesions.

In the present trial, nano-hydroxyapatite gel was found to have the highest significant effect on initial caries as compared to both fluoride varnish and TCP paste. This highly significant effect of nano-hydroxyapatite gel may be attributed to the high potential uptake of nano particles of hydroxyapatite by porous demineralized tooth structures. This finding was consistent with the results obtained by (Pepla et al., 2014) and (Sharma et al., 2017). This highly remineralizing effect may be explained by the small size of nano-hydroxyapatite crystals (50–1000 nm), which act as a filler for the small holes of the demineralized enamel surface. These results were in consonance with the findings of (Kamath et al., 2017).

A comparison between TCP and nano-hydroxyapatite demonstrated the nano-hydroxyapatite was more effective than TCP, which is inconsistent with the results obtained by (Kamath et al., 2017). This disagreement could be attributed to the difference in the study designs of both studies, because the in vitro study can be controlled for all variables. On other hand, it is difficult to control all the variables (randomized) in the in vivo study.

This study found a lack of standardized diet and oral hygiene measures, which may act as confounding factors affecting the results. Therefore, our recommendation is that future studies should be conducted with the standardization of diet and oral hygiene measures.

5. Conclusion and recommendation

The present study concluded that the three remineralizing agents were indeed effective in the treatment of initial carious lesion and that the most effective remineralizing agent was nano-hydroxyapatite, which is why it is highly recommended. However, further clinical trials are recommended in future in order to evaluate the effect of these agents for long periods of time.

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Declaration of conflict of Interest

The authors declare that there is no conflict of interests.

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