Journal of Research in Medical and Dental Science
2022, Volume 10, Issue 9, Page No: 107-112

Available Online at: www.jrmds.in
eISSN No.2347-2367: pISSN No.2347-2545

Comparative Evaluation of Remineralization of White Spot Lesion in Nano Silver Fluoride and Nano Hydroxyapatite Varnish: An *In vitro* Study

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ABSTRACT

Introduction: White spot lesions are a common finding with orthodontic treatment. Proper maintenance of oral hygiene, regular scaling, usage of fluoridated toothpaste, application of varnish etc. have been used to prevent, reduce or re-mineralize white spot lesions. Unfortunately, a method to completely prevent white spot lesions has yet to be achieved.

Aim: To determine and compare the efficacy of re-mineralization of white spot lesions between two green synthesized nanoparticle varnishes namely nano silver fluoride and nano hydroxyapatite varnish.

Materials and methods: 30 samples of extracted human premolar teeth were subjected to demineralization solution for 72 hours to artificially induce an incipient lesion. They were randomly divided into 3 groups 1) Green synthesized NaF; 2) Green synthesized HAP; 3) Control (no treatment). A total of 9 random samples were selected and subjected to Vickers microhardness test and an atomic force microscope to analyse the surface micro-hardness test and analysed with one way ANOVA and repeated measures ANOVA. Level of significance was set at p=0.05.

Results: At T_1, the surface micro-hardness test values of all samples was not statistically different among the groups (p=-0.8). Mean of surface micro-hardness of induced white spot lesion was 125.7 ± 16.5 were significant than baseline surface micro-hardness test values (p<0.001). The highest surface micro-hardness test values were observed in green synthesized sodium silver fluoride group (mean 192.6 ± 29.86). The surface micro-hardness of green synthesized HAP was 171.5 ± 20.66 and that of the control group was 113.2 ± 19.3. Intergroup analysis shows no significant difference between green synthesized sodium silver fluoride and green synthesized hydroxyapatite (p=0.001).

Conclusion: Green synthesized sodium silver fluoride n-NSF can be considered as an option for arresting white spot lesions in orthodontic patients. However, more in-vivo studies are needed to review the long term effects of the varnish.

Key words: Nanoparticle varnish, Caries prevention, Sodium fluoride, Hydroxyapatite, White tea

HOW TO CITE THIS ARTICLE: Rama Raji Sankaranarayanan, Navaneethan R, Comparative Evaluation of Remineralization of White Spot Lesion in Nano Silver Fluoride and Nano Hydroxyapatite Varnish: An *In vitro* Study, J Res Med Dent Sci, 2022, 10 (9): 107-112.

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E-mail: dr.ramarajis@gmail.com
Received: 28-Jun-2022, Manuscript No. JRMDS-22-50318;
Editor assigned: 30-Jun-2022, Pre QC No. JRMDS-22-50318 (PQ);
Reviewed: 14-July-2022, QC No. JRMDS-22-50318;
Revised: 29-Aug-2022, Manuscript No. JRMDS-22-50318 (R);
Published: 05-Sep-2022

INTRODUCTION

White spot lesion is defined as "white opacity", occurring as a result of a subsurface enamel demineralization that is located on the smooth surface of teeth [1]. The white opacity is the change in the light scattering optical properties due to demineralization of the enamel [2]. Fermentable carbohydrates, acid producing bacteria, poor oral hygiene, low pH of the saliva and diet high in sugar contribute to such incipient lesions [3]. Many research shows that white spot lesions develop due to long standing undisturbed plaque accumulation [4,5]. This creates an environment which leads to decrease in pH, where the acid penetrates underneath the plaque causing demineralization of the enamel followed by cavitation which can occur in 4 weeks [6,7].

Studies have explained the pathophysiology of caries where there was presence of bacteria in an acid environment in the plaque or biofilm [8,9]. Orthodontic brackets are one of the most important factors of food and debris lodgement which may tend to aggravate the white spot lesion. It becomes a high priority to maintain oral hygiene [10-12]. After the removal of these brackets and bands there is remineralization of the lesion which becomes less opaque but not completely. Hence, it becomes really important for us to arrest such lesions in the beginning [14].

Fluoride varnishes have been widely used to arrest enamel lesions with formation of Fluor apatite [15]. Varnish is preferred over other methods due to increased contact between teeth and varnish [16]. This forms a protective covering over the teeth thus increasing the prophylactic effect of the varnish. Nanotechnology is the new emerging field of science where the particles are microsized measured in nanometres. Nanoparticle was first introduced by Nobel laureate Richard P. Feyman in his famous lecture in 1959: “There’s plenty of room at the bottom” [17]. Nanoparticles are substances which are less than 100 nm. These are available in 0D, 1D, 2D, 3D depending on the shapes [18]. In recent times, nanoparticles have been incorporated in dental varnish which increases the ability to penetrate deep into the enamel surface rather than just coating superficially. Considering the toxicity of synthetic particles nanoparticles have been created from naturally occurring *Camelia sinesis* (White tea) incorporated with sodium silver and hydroxyapatite particles [19].

MATERIALS AND METHODS

This *in-vitro* study was approved by the university ethical committee. The study used sound extracted human premolar teeth from patients who underwent orthodontic treatment. Sample size was N=30 which was calculated using G power software, post hoc test. Teeth with enamel defects and decay were excluded from the study [20]. The specimens were washed and disinfected in 0.1% thymol. They were then mounted on epoxy resin such that the buccal surface was exposed (Figure 1). Later, tooth mounted resin models were polished with silicon carbide bur (2400 grit) and washed in distilled water for 20 seconds.

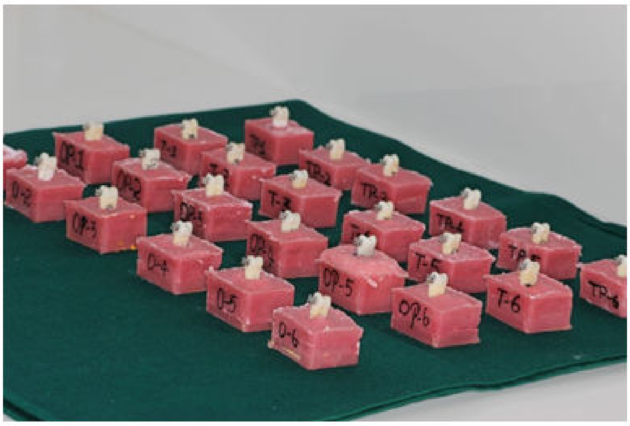

Figure 1: Specimens mounted on epoxy resin.

The baseline surface micro-hardness test (T_1) was done using Vickers microhardness tester (Mitutoyo HM100, Japan) (Figure 2) under 50 grams load for 10 seconds. Surface microhardness test was analysed at 4 different random points on the crown surface. The first indentation was at the centre of the enamel and the other points anywhere around at a distance of 300 um. Surface Microhardness test (SMH) was performed by a single operator and samples were selected whose SMH was in the range of 262 to 389 according to ten cates. Samples which had SMH below this were excluded from the study [21].

Figure 2: Specimens loaded for Vickers microhardness test 50 grams for 10 seconds.

Production of artificial carious lesion (T_2)

The demineralizing solution (Figure 3) was prepared with 50 mM acetic acid, 2.2 mM potassium dihydrogen phosphate, 2.2 mM calcium nitrate and 0.1 ppm NaF and the specimens were immersed in the solution for 3 hours. After this the specimens were immersed in a remineralization solution containing CPP-ACP for 21 hours. It was allowed to air dry after which Ormco brackets were bonded to the sample teeth using an orthodontic adhesive. This was followed with a surface microhardness test (T_2).

Figure 3: Preparation of demineralizing solution.

The 30 samples were divided into 3 groups with 10 in each group and were subjected to their respective varnish application protocols as follows [22].

Group 1: Control group (where no varnish application was done).

Group 2: Green synthesized Sodium silver fluoride (n-NaAgF) nanoparticle varnish (Figure 4) was applied with a sponge for three minutes over the specimens. After this, the varnish was kept in contact with the enamel surface of the specimens for 20 minutes. Then the samples were washed for 20 seconds with deionized water [23].

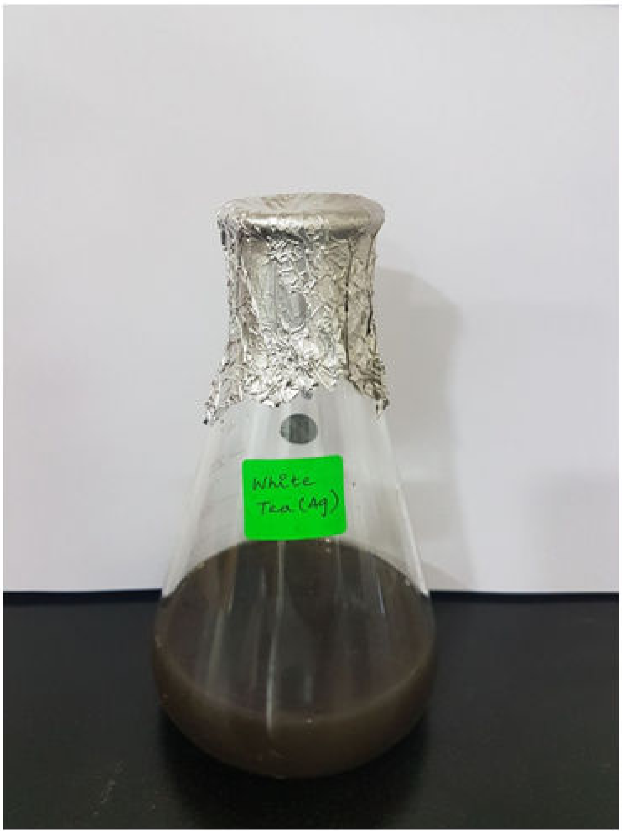

Figure 4: Preparation of Nano Sodium silver fluoride (n-NaAgF) from Camelia sinesis.

Group 3: Green synthesized hydroxyapatite nanoparticle varnish (n-HAP) (Figure 5) The application of the n-HAP varnish with a brush on the tooth surface and allowed to dry. The samples were then subjected to demineralization solution for 3 hours and remineralization solution (CCP-ACP) for 21 hours. This cycle of immersion in demineralization solution followed by immersion in remineralization solution was continued daily for one month with the solutions being made fresh every 3 days. The application of the n- HAP varnish was repeated every 10 days during this one month period [24].

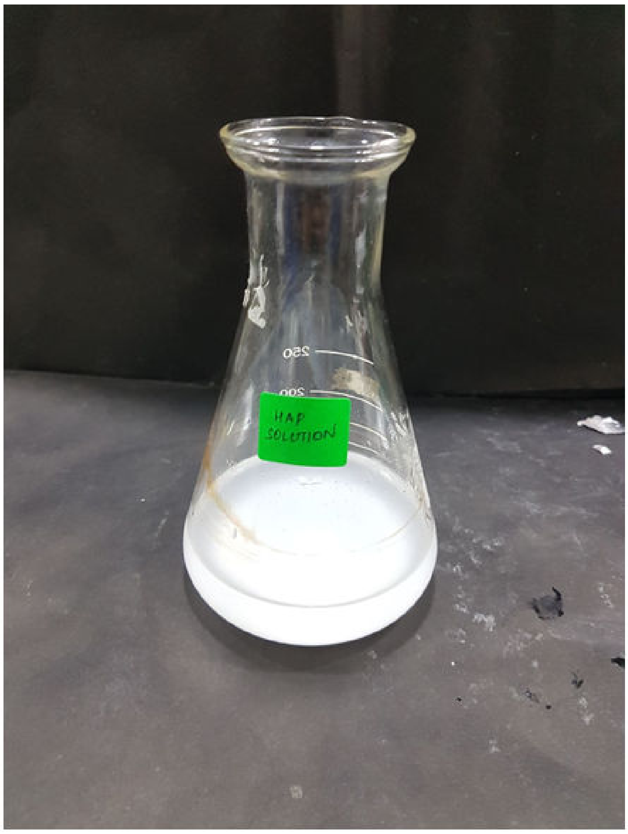

Figure 5: Preparation of nano hydroxyapatite (n-HAP) from *Camelia Sinesis.*

Following the respective varnish treatment procedures, the surface microhardness test was done for the samples of the three groups and the values were determined (T_3).

Statistical analysis: The power of the study was calculated using post-hoc in G power software to N=30. Statistics were calculated using the SPSS version 17 (IBM®, Chicago, IL, USA). The Shapiro-Wilk test confirmed the data distribution normality [25]. The surface microhardness test values and the surface microhardness recovery percentage were compared at three time intervals-T_1, T_2, and T_3 with One-way ANOVA and also the Sidak Post-hoc test. Repeated measures ANOVA and the Tukey HSD Post hoc test were used for inter-group comparisons. The level of significance was set at 0.05.

RESULTS

The T_1, T_2 and T_3 microhardness test for each group is shown in Table 1. At T_1, the surface micro-hardness test values of all samples ranged from 262 to 389 (mean 330 ± 25.06) which was not statistically different among the groups (p=0.8). Surface microhardness test values of T_2 ranged from 115.4 to 125.6 (mean 125.7 ± 16.5), were significantly lesser than the T_1 surface micro-hardness values ($p<0.05$). The highest surface microhardness test values at T_3 were observed in green synthesized sodium silver fluoride group (mean 192.6 ± 29.86) followed by that of green synthesized HAP (mean value: 171.5 ±

20.66) while the control group had a mean value of 113.2 ± 19.3.

Table 1: Mean Surface microhardness values of the three groups at T_1, T_2 and T_3. (Sidak Post hoc test).

	T_1	T_2	T_3
CONTROL	333.1 ± 26.03	125.6 ± 17.2	113.2 ± 19.3
n-NSF	331.2 ± 26.3	115.4 ± 11.5	192.6 ± 29.86
n-HAP	330.4 ± 20.4	125.6 ± 11.8	171.5 ± 20.66

Intergroup analysis with post hoc Tukey HSD test has been shown in Table 2. There was a significant difference between green synthesized sodium silver fluoride and green synthesized hydroxyapatite (p=0.001). The surface hardness recovery was observed in both the treatment groups but not in the control group [26]. The results of both the interventional groups revealed significant differences between the groups except for the control group (p=0.8).

Table 2: Comparison between groups of post-treatment surface microhardness values with repeated measure ANOVA and post-hoc Tukey HSD test

	n-HAP	NSF	CONTROL
n-HAP	p=0.2	p<0.05	p<0.001
n-NSF	p<0.001	p=0.7	p<0.001
Control	p<0.001	p<0.001	p=0.8

level of significance p<0.05.

DISCUSSION

The prevention of white spot lesion has been significance in orthodontics. Identification of early carious lesions and its prevention has led to development of many non-surgical methods 1920**.** Susceptibility of retention of plaque in orthodontic brackets is common. Diet, oral hygiene, frequency of eating has been the most important factors causing white spot lesions [21]. In a study by Derks, et al. he concluded that most orthodontists (95%) only provide oral hygiene instructions and around 50% provide fluoride mouth rinses. Hence all these factors increase the prevalence of white spot lesions among orthodontic patients and demands effective in office and at home oral hygiene [27]. This study demonstrates the efficacy of two nanoparticle varnishes namely green synthesized sodium Silver Fluoride (NSF) and green synthesized hydroxyapatite varnish for prevention of white spot lesions. Elements such as silver, gold, zinc oxide and chitosan have antimicrobial property against streptococcus mutans [23,24] which has led to development of formulations for anticaries activity [28].

Sondi et al. concluded that silver nanoparticles can cause structural changes and lead to bacterial cell death by penetrating the bacterial cell wall. Haghoo et al. had suggested that NSF could be a promising agent against Streptococcus Mutans and Streptococcus salivarius. Santos et al. compared Nano Silver Fluoride (NSF) with Silver Diamine Fluoride (SDF) for efficacy in arresting incipient caries in pedodontic patients. In their study, 66.7% of the lesions in teeth treated with NSF were arrested while only 34.7% treated with SDF were arrested. It was also found that SDF causes blackish discoloration of the teeth after remineralization due to deposition of silver particle penetration whereas this was less with Sodium silver fluoride varnish. In our study, the remineralization potential was highest with n-NSF group further attesting the efficacy of NSF in remineralization of enamel [29].

In this study we performed a green synthesis of n-NSF from *Camelia Sinesis* (White tea). Jose et al. showed that out of 21 plants used in their study, white tea (-87%) was found to have the highest anti-collagenase, anti-elastase and anti-oxidant activities followed by green tea (-47%), rose tincture (-41%) and lavender (-31%). Both the green tea and white tea have catechins like particles. In a study by rose et al. it has been found that the calcium in CPP-ACP binds to the plaque and doubles the availability of calcium and thus may aid in greater remineralization. Hence this was used in our study to increase the availability of calcium ions for remineralization. The results of this *in vitro* study revealed that green synthesized nano silver fluoride had a higher efficacy of remineralization compared to Nano hydroxyapatite varnish synthesized from *Camelia sinesis*. The effectiveness of remineralization of green synthesized n-silver fluoride and n-hydroxyapatite were similar with the other studies where silver fluoride varnish was compared with hydroxyapatite varnish [29-31]. de Carvalho FG, et al. had demonstrated similar remineralization results with nano hydroxyapatite. Aykildiz, et al. compared the remineralizing capacity among sodium silver fluoride, SDF and NaF and they found sodium silver fluoride to have the highest remineralizing capacity which was similar to our results. Comar et al. concluded that fluoride (0.2% NaF) was better in remineralization compared n-HAP and n-HAP pastes where they had compared the prevention of demineralization rather than the remineralization efficacy [30]. Tschoppe P, et al. concluded that nano hydroxyapatite was more effective than amine fluoride. However they had used n-HAP toothpastes with different formulations. This might have caused a difference in the results. They had also recommended usage of n-HAP as an alternative for candidates with fluorosis. Zhi QH et al. showed that both silver and fluoride were responsible for enamel remineralization and also demonstrated that silver can penetrate into those carious lesions and arrest the lesions [31]. The limitation of the current study is that it was an *in vitro* study, the effects of the varnish in an *in vivo* set up has yet to be studied to check for other possible beneficial/adverse effects of the varnishes and also validate the current results. Hence further studies to determine the long term effects in an *in vivo* setup of these two varnishes are necessary [32].

CONCLUSION

Green synthesized nano sodium silver fluoride and nano hydroxyapatite varnishes can be used for remineralization of incipient and white spot lesions that occur as a result of orthodontic treatment. The remineralization efficacy of nano silver fluoride was found to be higher than that of nano-hydroxyapatite.

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