COMPARISON OF EFFICACY OF THREE DIFFERENT TYPES OF VARNISHES IN PREVENTING ENAMEL DEMINERALIZATION AROUND ORTHODONTIC BRACKETS- AN INVITRO STUDY

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Abstract

Introduction: Formation of White Spot Lesions is one of the common side effect during long term Orthodontic treatment. Accumulation of plaque and calculus around the bracket is the key factor for its formation. There are several ways to control its formation. Present invitro study evaluates the efficacy of three different types of varnishes in preventing enamel demineralization around orthodontic brackets.

Methods: 80 freshly extracted premolars were collected for the study. After inclusion and exclusion criteria 72 teeth were selected and divided into 4 groups with 18 teeth in each group. Group 1 is control were no varnishes is applied, Group 2 contains teeth which was coated with 5% sodium fluoride varnish (voco proflurid), Group 3 contains teeth which were coated with ACP-CCP varnish (GC MI), Group 4 contains teeth which were coated with Nano silver fluoride varnish (NSF). After demineralization cycle the teeth were ground sectioned using hard tissue microtome. The teeth were then analyzed under polarization microscope for the depth of enamel demineralization.

Results: Increased amount of enamel demineralization was found in Control group (32.26±11.61µm) Followed by Group 2 (27.19± 13.15µm) , Group 3 (28.22±14.5µm). The Group 4 showed least amount of enamel demineralization (12.1±5.1µm). There was a statistically significant difference in the measured depths of enamel demineralization in control group when compared with other groups (p=0.00).

Conclusion: The NSF group (group 4) showed least amount of depth of enamel demineralization. Hence Nano Silver Fluoride can be used as varnish for preventing formation of white spot lesions around Orthodontic brackets instead of conventional fluoride varnishes.

Keywords: White Spot Lesions, Nano Silver Fluoride, Enamel Demineralization.

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Introduction:

The comprehensive Orthodontic treatment is a lengthy procedure and this leads to iatrogenic consequences such as root resorption, plaque-induced demineralization and white spot lesions which leads to Orthodontic scars.[1]

White Spot Lesions (WSL) appears as a small or large decalcified areas around the brackets with or without cavitation and have a chalky white appearance. These lesions are caused by organic acid produced by biofilms around Orthodontic brackets, which in turn demineralize the enamel.[2]

The morphology of fixed Orthodontic appliances results in stagnation of plaque and impede plaque removal during tooth brushing and natural self-cleaning mechanisms.[3] White spot lesions can develop around brackets as early as 4 weeks after the initiation of Orthodontic treatment, and prevalence rates as high as 73.5%[4] among patients undergoing Orthodontic treatment have been reported. Therefore, inhibiting the development of carious lesions around Orthodontic appliances is important. Many methods were found to be effective in decreasing white spot lesions; by improving oral hygiene, modifying diet (low carbohydrate), and treating with topical varnishes.[5]

There are many topical re-mineralizing agents that have been used to prevent demineralization and re-mineralize the enamel. Fluoride is known to control caries predominantly through its topical effect of inhibiting demineralization by forming fluorapatite crystals on the enamel surface.[6] Currently, casein phosphor-peptide and amorphous calcium phosphate are also used as re-mineralizing agents.[7] Casein phospho-peptides (CPP) binds to amorphous calcium phosphate, forming small clusters of casein phosphor-peptide-amorphous calcium phosphate (CPP-ACP), thereby stabilizing calcium phosphates in solution.[8]

With the advent of nanotechnology, silver nanoparticles (AgNPs) have been synthesized, and they have shown potent antimicrobial properties.[9]

The antimicrobial mechanism of AgNPs has been attributed that silver ions interact with the peptidoglycan cell wall causing structural changes.[10] In turn NSF as a varnish has anticariogenic property due to the presence of chitosan and fluoride present in it. Present study was done to compare and evaluate the effectiveness of three different types of varnishes with control group in preventing demineralization of enamel surrounding the bonded Orthodontic brackets.

Materials and methods:

The present study was conducted at Sri Siddhartha dental college and hospital, Agalakote, Tumkur after the approval from the institutional ethical committee (SSMC/Dent/IEC-5/Dec2020).

Eighty Freshly extracted premolars were collected and stored in distilled water. After the collection of samples- decayed, attrited and restored teeth were excluded. The teeth were thoroughly cleaned and washed under running water to remove all adherent soft tissues (Figure 1).

Figure 1: Extracted premolar teeth

After cleaning and excluding the damaged teeth Seventy-two samples were finalized for the study.

The samples were stored in distilled water for 5 days, soft tissues, calculus, debris present over the teeth were removed using scaler (Woodpecker) priorly. All the sample teeth were etched (37% phosphoric acid, Ivoclar Vivadent N-tech) and 3M unitec brackets were bonded using composite resin (Enlight, Ormco) to the buccal surface of the teeth and flash was removed.

Light curing was done for 3 sec using high intensity Woodpecker I- LED light curing unit. After bracket bonding, samples were randomly divided into four groups consisting of 18 samples in each group (Figure 2).

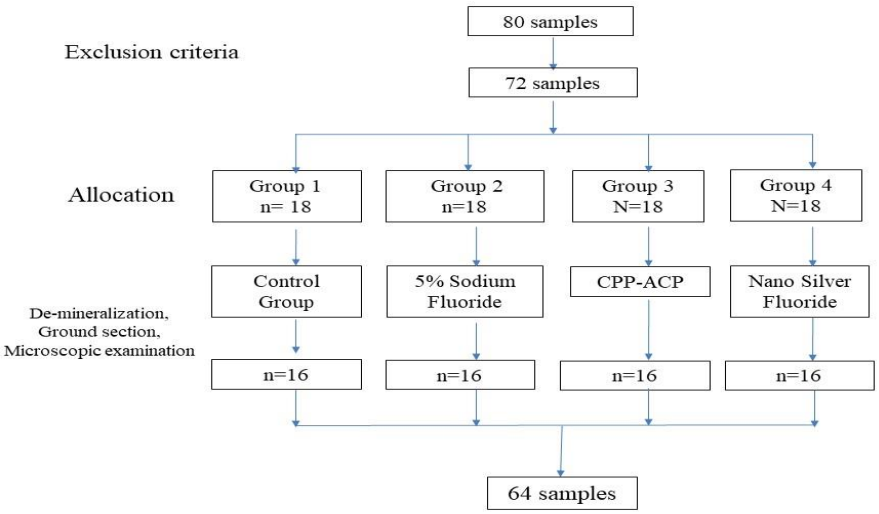

Figure 2- Design of the study

All the teeth were painted using nail varnish except the buccal surface to avoid weakening of teeth and allowed it to dry for 2 days. All the samples were applied with respective varnishes except the control group. The Four groups were as follows (Figure 3):

Group 1 (n=18): The teeth were not coated with any varnish (Control group).

Group 2 (n=18): The teeth were coated with 5% Sodium fluoride (VOCO Profluorid[tm])

Group 3 (n=18): The teeth were coated with Fluoride with Amorphous calcium phosphate - Caesin phosphor-peptide ACP-CCP (GC MI Varnish, Recaldent [tm])

Group 4 (n=18): The teeth were coated with Nano Silver Fluoride varnish (AgF, 99.9%, APS<10nm) (NANOrh[tm]).

Figure 3 - Varnishes used in the study

Preparation of demineralizing solution

The demineralizing solution was prepared using 2.2 mM calcium chloride, 2.2 mM potassium hydrogen orthophosphate, unstirred solution of 0.05 M acetic acid, and 1M potassium hydroxide (KOH) pH at 4-5. Preparation of demineralizing solution is done by mixing 2.2 g of calcium chloride in 1050 ml of distilled water in a beaker. 2.2g potassium hydrogen orthophosphate, 3g of acetic acid, and 56g of KOH were added to the solution.[11]

The teeth were then placed in artificial saliva for 3 days and the specimens were subjected to demineralization cycle by immersing in prepared demineralizing solution with a final pH of 4 for 96 hr. at normal room temperature to produce demineralization. The pH was maintained at 4 by a trained technician. Then all the teeth were rinsed using distilled water. The brackets were de-bonded and then they were mounted over the acrylic block. Using ISOMET 1000 Precision saw the teeth were ground sectioned buccolingually in the middle third of the tooth longitudinally in a very thin section up to 150 microns (Figure-4). At the end of sectioning each group was remained with 16 samples. Then the sectioned tooth was rinsed using absolute alcohol, xylene to remove any water molecules and mounted over the slide using DPX (Dibutyl phthalate Polystyrene Xylene) solution and the cover slip was placed over it.

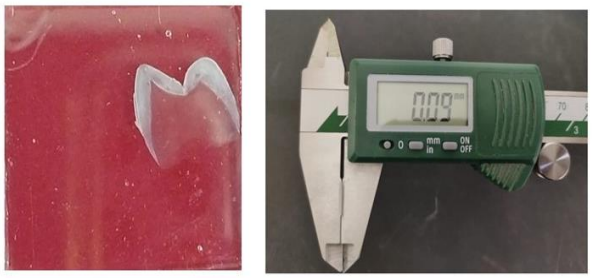

Figure 4-Buccolingual ground Sectioned teeth

Viewing under polarized light microscope

The sectioned teeth were evaluated under Polarization microscope (Olympus microscope BX43) under 10X magnification and the depth of enamel demineralization was measured using

image analysis software (ProgRes capture Pro V2.8.8, JENOPTIK) and was analyzed to compare the efficacy of each different varnishes in preventing enamel demineralization. The polarized microscopic images with measurements are shown in (Figure-5,6,7,8).

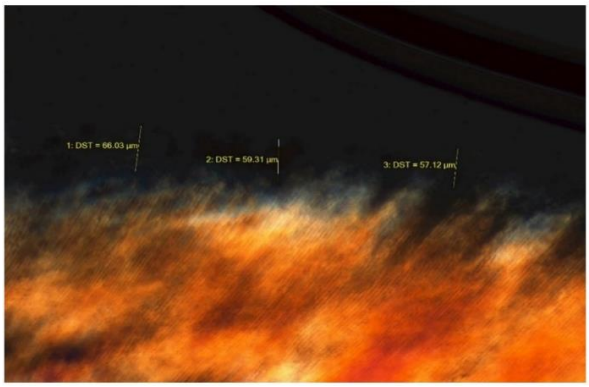

Figure 5 – Area of demineralization in Group 1 (control)

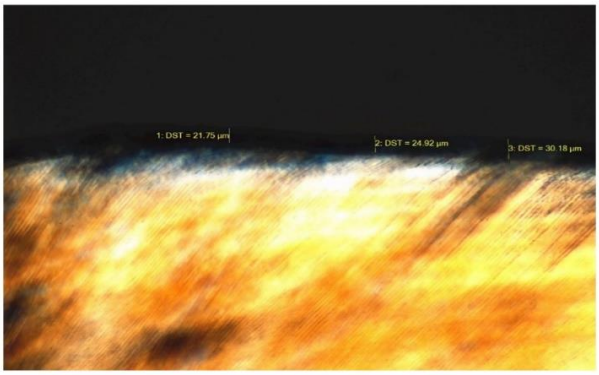

Figure 6 – Area of demineralization in Group 2 (5% Sodium Fluoride)

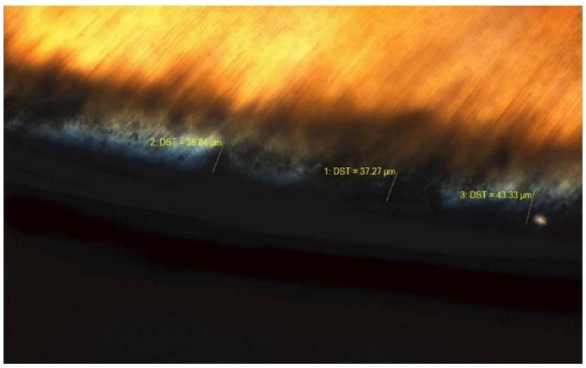

Figure 7 – Area of demineralization in Group 3 (CCP- ACP)

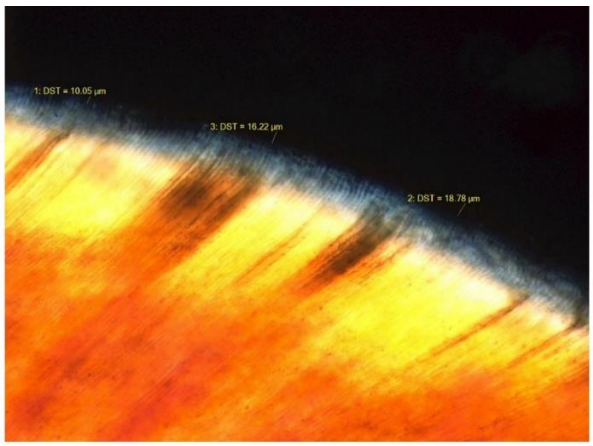

Figure 8- Area of demineralization in Group 4 (Nano silver fluoride)

Statistical analysis:

The data was processed and analyzed using the IBM Statistical Packages for Social Sciences, SPSS software version 23. Shapiro-Wilk test was used to test for normality of the data.

Results of continuous measurements measured as mean and standard deviation. ANOVA test and Dunn-Bonferroni post hoc test were used to compare the depths of enamel demineralization in different groups. p-value of less than 0.05 is considered statistically significant.

Results

Present study evaluated the depth of demineralization in different varnishes group compared with control group.

Table 1 illustrates the descriptive statistics for measured depths of enamel demineralization in different groups. The mean measured depths of enamel demineralization was lowest for group 4 (12.1±5.1µm) followed by group 2 (27.19± 13.15µm) and group 3 (28.22±14.5µm).

The control group (32.26±11.61µm) has the highest mean measured depths of enamel demineralization.

Table 1: Descriptive statistics for measured depths of enamel demineralization in different groups

Group	N (nos)	Mean (µm)	Std. Deviation (µm)	Minimum (µm)	Maximum (µm)
Control group (GROUP 1)	16	31.70	12.70	14.28	60.82
Experiment group 2	16	27.19	13.15	7.27	41.33
Experiment group 3	16	28.22	14.50	12.71	56.44
Experiment group 4	16	*12.10*	*5.10*	*5.74*	*26.15*
Total	64	24.80	13.90	5.74	60.82

Table 2: illustrates the intragroup comparison of depths of enamel demineralization. There was a statistically significant difference in the measured depths of enamel demineralization in control group when compared with other groups (p=0.00).

Table 2: Comparison of measured depths of enamel demineralization in different groups			
Groups	**Depth of enamel demineralization (Mean ± SD) (µm)**	**Z**	***P***
Control group (GROUP 1)	*31.7±12.7*	8.466	0.00* Significant
Experiment group 2	27.19±13.15		
Experiment group 3	28.22±14.5		
Experiment group 4	12.1±5.1		
ANOVA Test; * Statistically significant; p<0.05; NS- not significant			

Table 3 illustrates the comparison of measured depths of enamel demineralization of control group with other groups. When control group was compared individually with other groups, there was a statistically significant difference in the measured depths of enamel demineralization between control group and Group 4 was observed (p=0.01) (Figure 9).

Table 3: Comparison of measured depths of enamel demineralization between control group and other groups			
Groups		**Mean difference (µm)**	**P**
Control group (GROUP 1)	**Experiment group 2**	4.50	0.51 NS
	Experiment group 3	3.48	0.98 NS
	Experiment group 4	19.60	0.01* Significant
Dunn-Bonferroni post hoc test; *Statistically significant, p<0.05, NS- not significant			

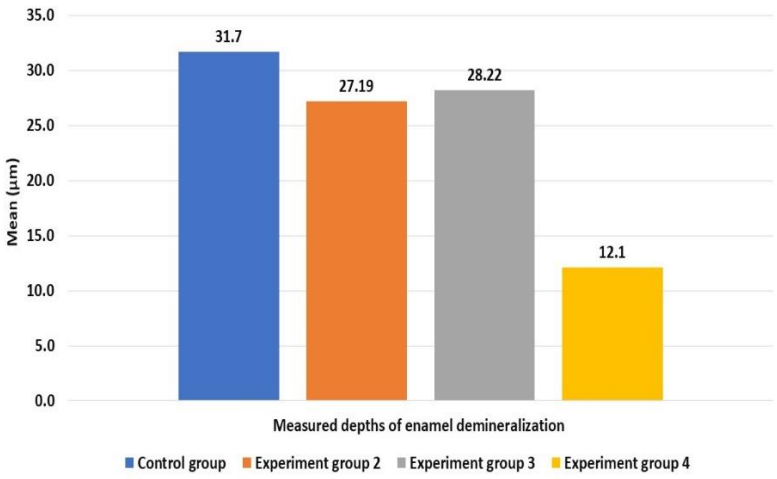

Figure 9- Comparison of depth of enamel demineralization between the groups

Discussion:

The white spot lesion is a white patchy appearance that can be formed as a result of enamel demineralization and the dissolution of hydroxyapatite as a result of acidic by-products of the bacteria in the dental plaque. The formation of white spot lesions can be prevented by proper maintenance of oral hygiene. Other methods of preventing its formation includes application of varnishes, bonding brackets using fluoride releasing composites, mouth washes etc.[12] Previous studies have illustrated that 5% sodium fluoride varnish works well in preventing WSL formation.[13] Other varnishes such as CPP- ACP, Silver diamine fluoride, Nano silver fluoride also showed considerable prevention against WSL formation. Since NSF is a good antimicrobial agent it also functions as re-mineralizing agent.[14,15] Many studies have been done to evaluate its anti-microbial and re-mineralizing properties of NSF.[16] Present study evaluated three different varnishes namely 5% sodium fluoride, casein phosphopep tide- amorphous calcium phosphate, Nano silver fluoride compared with Group 1 (control group) in prevention of enamel de-mineralization around Orthodontic brackets. Present study showed that the mean measured depths of enamel demineralization was lowest for Group 4 (12.1±5.1µm) followed by Group 2 (27.19±13.15 µm) and Group 3 (28.22±14.5 µm). The control

group (32.26±11.61 μm) had the highest mean measured depths of enamel demineralization which was subjected to surface demineralization without applying any varnishes. The cariogenic solution was maintained at pH 4 and was repeatedly checked by a trained technician. The single application of 5% sodium fluoride in present study showed significant amount of reduction of enamel demineralization (27.19±13.15μm) which is similar to the study done by Todd et.al[17] where treated group (5% sodium fluoride) showed 50% less enamel demineralization when compared to non-treated control groups. Demito et.al[18] reported there was 38% reduction in the mean depth of enamel demineralization lesions adjacent to Orthodontic brackets applied with Duraflor fluoride varnish. This reveals that there will be no complete prevention of formation of white spot lesions moreover it reduces its formation. Ogaard et. al[19] reported that it was due to formation of calcium fluoride on the enamel surface which in turn helps in the formation of fluorapatite crystals that prevents further enamel demineralization. The casein phosphopeptide- amorphous calcium phosphate (CPP-ACP) group in the present study showed significantly less depth (28.22±14.5 μm) of enamel demineralization, whereas previous studies by Bichu et al[14] had reported that reduction in demineralization was up to a mean depth of 80.5μm. Similar reduction in depth of enamel demineralization was reported in other studies by Sudjalim and nabbas[20] Recent study done by Moufida Abufarwa et. al[21] had evaluated the samples which were treated using CPP-ACP varnish after etching showed increased micro hardness when compared to other groups. The reason behind the preventive and re-mineralizing property of CPP-ACP was attributed to high amount of release of calcium and phosphate ions and immediate reaction over enamel surface and formation of calcium fluoride which acts as reservoir for fluoride release and it significantly increasing the micro hardness of the enamel surface. Present study had showed its effect in reducing the depth of demineralization.

Though many studies have evaluated the remineralizing and anti-microbial properties of NSF, very few studies elaborated its anti-carious properties. Present study showed reduced depth of enamel demineralisation (12.1±5.1μm) which is similar to studies done by El-Desouky et. al.[22] The reason behind this was attributed to the smallest particle size and new experimental formulation which contains AgNPs, chitosan and fluoride present in it. The potential agent used in the current caries arrest formulation is chitosan, a polysaccharide composed of glucosamine copolymers and *N*-acetyl glucosamine.

The resulting polycation is soluble in aqueous solutions of small organic acids such as acetic acid and lactic acids and can be linked in the presence of polyvalent anions such as phosphates. Many in vitro studies have shown that chitosan interferes with demineralization of the tooth enamel inhibiting the release of mineral elements.[23] Tencate[24] stated that fluoride absorbed by demineralized dental hard tissue along with calcium and phosphate is a crystalline structure (remineralization) that will be more resistant to bacterial acid challenges.

When the mean depth of demineralization was compared between the groups, there was a statistically significant difference in the measured depths of enamel demineralization amongst all the three groups. The mean depth of demineralization was less for Sodium fluoride group (group 2) when compared with CPP-ACP group (group 3), but it was not significant. This result is not in congruent with the study done by Babu et.al[25] where CPP-ACP group showed increased surface microhardness. This could be attributed due to variance in the pH cycling method. The present study used pH cycling method proposed by TenCate and Duijsters.[26] Studies also showed CPP-ACP paste decreases lesion depth and has higher remineralization potential when it is used in combination with fluoride toothpaste than when used alone.

When 5% sodium fluoride group and Nano Silver Fluoride (NSF) group was compared in mean depth of demineralization, the NSF group showed reduced depth of enamel demineralization. This is in contrary with the other study done by El-Desouky et al.[22] where both NSF and 5% sodium fluoride showed similar effects in limiting enamel demineralization caused by artificial cariogenic challenge. Teixera et. al[15] also reported there was no significant difference between NSF and NaF in preventing demineralization of enamel surface.

When control group was compared individually with other groups, there was a statistically significant difference in the measured depths of enamel demineralization between control group and NSF Group (p=0.01).

The polarization microscope and software used in present study allowed for precise and direct measurements of demineralization depths, allowing the effects of various agents to be compared. This is critical for a realistic evaluation of any therapeutic intervention. The comparison of measurement depths in present study served as an indicator of the different agent's demineralization

inhibition potential. For all samples, the magnification was kept at 10X to show a clear area of interest.

One of the draw-back of the study was that in many samples, after debonding the bracket the composite had been remaining on the surface of the tooth so that it is difficult to approximate the site of interest for observing the depth of demineralization under polarization microscope.

As this study was carried out under in vitro conditions on premolar teeth, the results may not be transferred completely to an in vivo situation. The process of dental caries is dependent on several biological factors that are present in the oral environment. Further in-vivo studies must be done to evaluate the efficacy of different varnishes effectively.

Conclusion:

- There was a significant difference in mean depth of enamel demineralization between all the three groups.
- When compared with control only Nano silver fluoride varnish (Group 4) showed significant difference.
- The NSF group showed least amount of depth of enamel demineralization.
- Hence NSF can be used as varnish for preventing formation of white spot lesions around Orthodontic brackets instead of conventional fluoride varnishes.

Clinical significance:

Nano Silver Fluoride varnish can be used as an alternative for conventional varnishes due to its antimicrobial, re-mineralizing as well as inhibiting demineralization properties. Since nano particles has many hazards, its properties should be studied completely before made it commercially available.

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Conflict of Interest

Authors has no conflict of interest.

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